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Effect of gas barrier imperfections on Vitamin C deterioration in chilled orange juice stored in EVOH gable top cartons

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Andreas Fredrik Thoresen

ABSTRACT

Gas barrier imperfections in gable top cartons can lead to oxygen from the ambience permeating a carton, causing deterioration of vitamin C in fruit juices. Barrier imperfections in relation to vitamin C in orange juice have not yet been investigated in any scientific paper. Neither have imperfections in the gas barrier in Ethylene Vinyl Alcohol (EVOH)- or aluminum barrier cartons in relation to oxygen Transfer Rate (OTR) been investigated in a scientific paper.

In this thesis, gable top cartons with an EVOH or an aluminum gas barrier was investigated to find the effect barrier imperfections had on deterioration of vitamin C in chill stored orange juice. This project attempted to quantify and describe gas barrier imperfections in the gas barrier in gable top cartons with available methods and investigate if there was a relation to degradation of vitamin C in orange juice, and to develop a practical method for measuring OTR of a gable top carton with perforations with the Ambient oxygen Ingress Rate (AOIR)-method.

Perforations and barrier imperfections were generated in the gas barrier in gable top cartons and the cartons filled with juice. During storage for 8 weeks, sampling investigated headspace oxygen concentration, dissolved oxygen content in the juice and vitamin C deterioration. Cartons were also tested with dye-testing and investigated in microscope to describe gas barrier imperfections. Also, a practical method for measuring OTR of a gable top carton with the AOIR-method was developed.

Aluminum barrier cartons retained more vitamin C than EVOH barrier cartons. Some, but not all seal barrier imperfections affected vitamin C degradation in orange juice. Of the 7 different perforations generated in EVOH cartons, 4 did not have statistically different vitamin C content than a reference without perforations, after 8 weeks.

Dissolved oxygen does not equalize in orange juice, as there was measured a higher concentration in the top of the orange juice and less in the bottom, creating a gradient in the juice. The area of color penetration from a dye test did not correlate with vitamin C content or OTR. Neither did vitamin C deterioration correlate with OTR measured with AOIR. An EVOH gable top carton does not have to be free of barrier imperfections to retain vitamin C content in orange juice, as certain perforations did not cause greater deterioration of vitamin C. Further work is needed to complete and validate the AOIR method for use in Cartons.

1. INTRODUCTION

1.1 PURPOSE

This thesis aimed to associate the results of the most used methods in the industry to evaluate the quality of orange juice and the barrier integrity of gable top cartons, in addition to, developing a practical method for measuring Oxygen Transfer Rate (OTR) of a gable top carton using the Ambient Oxygen Ingress Rate (AOIR)-method.

This included investigating the effect of gas barrier imperfections on Vitamin C content development, headspace oxygen development and dissolved oxygen development in gable top cartons with an Ethylene Vinyl Alcohol (EVOH) and aluminum gas barrier filled with orange juice and stored in a refrigerated environment.

Developing a procedure for using Ambient Oxygen Transfer Rate (AOIR)-method, developed by Hanne Larsen and others in 2000 (Larsen et al. 2000), for measuring oxygen transfer rate (OTR) in gable top cartons with barrier imperfections. Also, discuss if AOIR is a suitable method for measuring OTR in gable top cartons with barrier imperfections.

Find if there is a correlation between carton headspace oxygen concentration, dissolved oxygen content and vitamin C content. Find if there is a correlation between vitamin C content in a carton or OTR measured with the AOIR-method and the current destructive liquid test method using ethanol and blue dye. Find if there is a correlation between vitamin C content in a carton and OTR measured with the AOIR-method.

The AOIR-method, headspace oxygen concentration measurement, dissolved oxygen concentration measurement, the dye test method and microscopy were used to relate properties of gas barrier damages to the deterioration of vitamin C in orange juice. Gas barrier imperfections in gable top cartons can lead to oxygen from the ambience permeating a carton and cause deterioration of vitamin C in orange juice. Barrier imperfections in relation to vitamin C in orange juice has not yet been investigated in any scientific paper published. Neither has imperfections in the gas barrier in EVOH- or aluminum barrier cartons in relation to OTR been investigated in a scientific paper (Larsen, Liland 2013).

There is an extensive amount of knowledge about barrier imperfections in gable top cartons in the R/D-departments at the packaging producers, but many of the effects has been observed and not documented in a scientific paper. The hypothesis being that there is a relation between character of gas barrier imperfections, headspace O_2, dissolved O_2, OTR, dye-penetration area and vitamin C content. If a correlation could be found between the values for each method and vitamin C content the methods for testing juice quality and carton

integrity could be further reassured. In the packaging industry, the financial and environmental aspect of the package is a driver to utilize the least amount of material to produce each package. This in turn makes the knowledge of the different package properties under different conditions crucial.

Quality testing tools for gable top cartons used today in the industry are visual inspections after dye testing or in a microscope. There is no documented knowledge if dye penetration area on the board in a carton can be used as a tool to assess the severity of a barrier imperfection. Dye testing is today used as a tool to identify if a barrier imperfection in a gable top carton is present after filling. OxTran is used to test permeation in gable top cartons today, but is limited to an OTR of 1.5 ml O_2/package/day (Sara Lisboa, Elopak, 8.12.16). Developing AOIR as a usable method for gable top cartons with high OTR (>1.5 ml O_2/package/day) could be an opportunity to describe the permeation in a carton with perforations, that perhaps does not affect vitamin C degradation. Vitamin C content measurements in orange juice is used today as a tool to determine orange juice quality and can reflect carton barrier quality. Although possible correlations between different quality testing methods are not known.

1.2 GABLE-TOP CARTONS

A gable top carton is built from two fundamental materials, wood fiber and polymers, which can undergo multiple production steps and treatments from raw material to finished product. The structure and strength is provided by a wood fiber board, commonly made from pine or spruce, but also other wood fibers. (Eie 2007). Fiber board can be prepared by mechanical or chemical processing of wood fiber, where mechanical processing can be assisted by steam or small amounts of chemicals. Mechanical processing can create a higher amount of damaged fibers and few free single fibers compared to a purely chemical process. Today the processing is adjusted to fit the result to the needed capabilities of the board (Eie 2007).

Different polymers have gas permeability properties ranging from high to low, thus the barrier and protective layers are chosen from different types of polymers, to achieve the desired barrier capabilities (Siracusa 2012, Hussein et al. 2015). Several layers of polymer can be included to provide the fiber board with protection from moisture and the product protection from ambient gases. The manufacturing process, by applying stress to the material can have an influence on the final permeability properties of a gable top carton, by causing gas barrier imperfections (Del-Valle et al. 2004, Siracusa 2012).

The most common polymer in a gable top carton is polyethylene (PE). The main purpose for using PE as a barrier in a gable top carton is as a liquid barrier and for sealing the carton due to melting properties (Eie 2007). Polyethylene subsist of nonpolar high molecular weight hydrocarbons, where the alignment of polymer chains relative to each other (crystallinity), determines density and chemo-mechanical stability (Carraher 2005). Non-polarity causes polyethylene to absorb almost no water but gases such as oxygen and carbon dioxide can pass a PE gas barrier with little resistance du to molecular structure (Eie 2007).

EVOH is a copolymer of ethylene and vinyl alcohol. EVOH is a strong barrier to oxygen that can be used as the gas barrier in a gable top carton. In a carton for storage of liquids, An EVOH-barrier must be protected between layers of PE, since the high amount of –OH-groups cause EVOH to interact with water. EVOH is a strong barrier against oxygen, nitrogen, carbon dioxide and helium (Hussein et al. 2015).

An aluminum barrier carton is a construction similar to an EVOH-carton, but where aluminum is used as the gas barrier. An aluminum barrier will have a low permeation, at a hundredth of that measured in an EVOH barrier carton (correspondence with Elopak employees).

The cartons used in the thesis was an EVOH-barrier carton and a carton with an aluminum-gas barrier. The EVOH carton consisted of several layers with various purposes. The outer layer of PE in a gable top carton is a protective layer for the board against moisture as well as a surface for printing information. The board layer provides strength and stability and defines the shape of the carton. The second layer of PE is the first layer on the inside of the carton and ensures all crevasses in the board are filled and that the surface is even for application of the remaining layers. The EVOH polymer layer is the gas barrier to prevent oxygen from entering the package. The innermost PE layer is the main moisture barrier.

The “aluminum” cartons used are also a construction of multiple layers. Several layers in the order of: PE, Aluminum, PE, board and PE, from the outside to the inside. In the aluminum carton, the aluminum has the function as a gas barrier. (Eie 2007)

A gable top carton production process follows several steps that can all have an influx on the properties of the final product. First the board is made from the pulp of pine, spruce or other wood fibers. The flat board can either be naturally brown like the wooden fibers, and provide natural sunlight protection, or it can be bleached. Bleaching of the board is when performed strictly for changing the esthetics of the carton. Next, multiple layers of polymer will be applied to both sides of the fiber board to provide the desired package properties. The desired carton blank shape is cut from the board to make the flat blank, illustrated in Figure 1.

with names for relevant structural components. This first step also uses a press to add creasing lines in the board to ease folding at later stages. The flat bank is then put through the first heating process in a converter illustrated in Figure 2. to weld the 1. panel to the 5. panel of the flat blank, either by heating with hot air or a gas flame.

Further production steps that may affect the blank structure will be carried out at the juice manufacturer in a filling machine, illustrated in Figure 3, where top seal heating is the most likely point of failure. Blanks will be inserted into a filling machine which will form, fill and seal a carton with a closure.

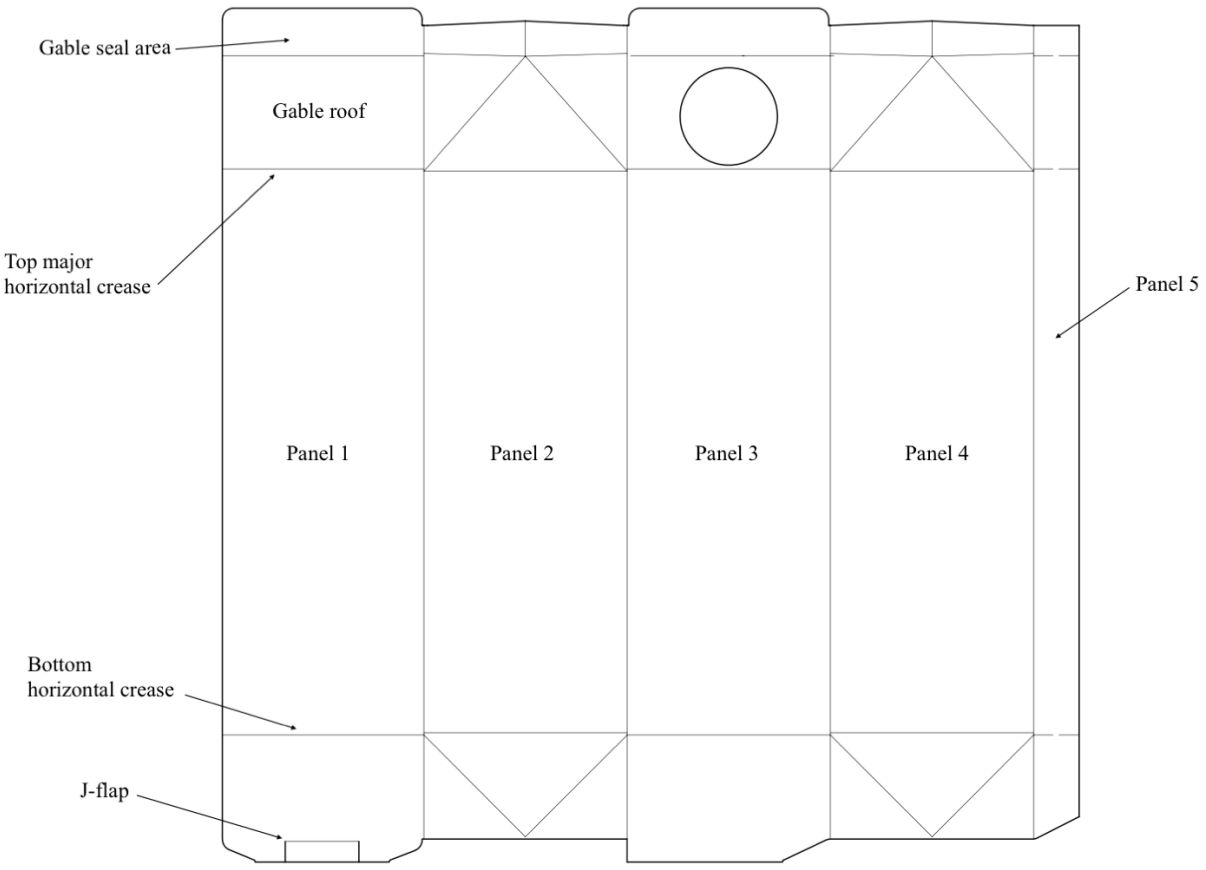

Figure 1: Illustration of a gable top carton flat blank. Terms for relevant structural components are indicated.

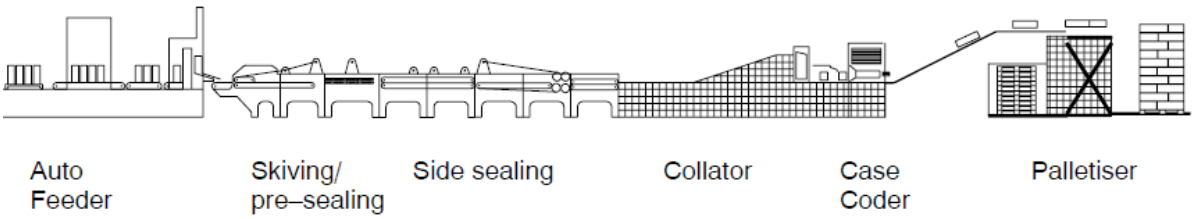

Figure 2: Illustration of a converter line for gable top carton flat blanks. A feeder moves flat blanks to a pre-sealing section where ½ of outer board along the fifth panel is removed and remaining polymer is folded around itself. In the side sealing section the 1. panel and 5. panel is joined by heating of PE and application of pressure. The collator applies pressure to the edges of the blank for pre-folding, as to ease processing in filling machine.

The filling process can be separated in seven steps; 1. Folding the blank open by applying pressure to the folded edges of the. 2. Fold the bottom edges to provide a flat bottom and weld with heated air. 3. Apply the closure and seal the closure with heat generated by ultrasonic vibration. 4. Flush the carton with hot hydrogen peroxide (H_2O_2) to disinfect the carton. 5. Fill the carton with product. 6. Heat the top seal of the carton with hot air. 7. Fold and seal the top of the gable on the carton with a water-cooled press. The full production process submits the carton blank to stress and can under the unideal conditions provide a carton that has damages in the barrier layers. Even under the right conditions, microscopic holes can form in folds and welding areas. These and other perforations will from here on now be referred to as barrier imperfections.

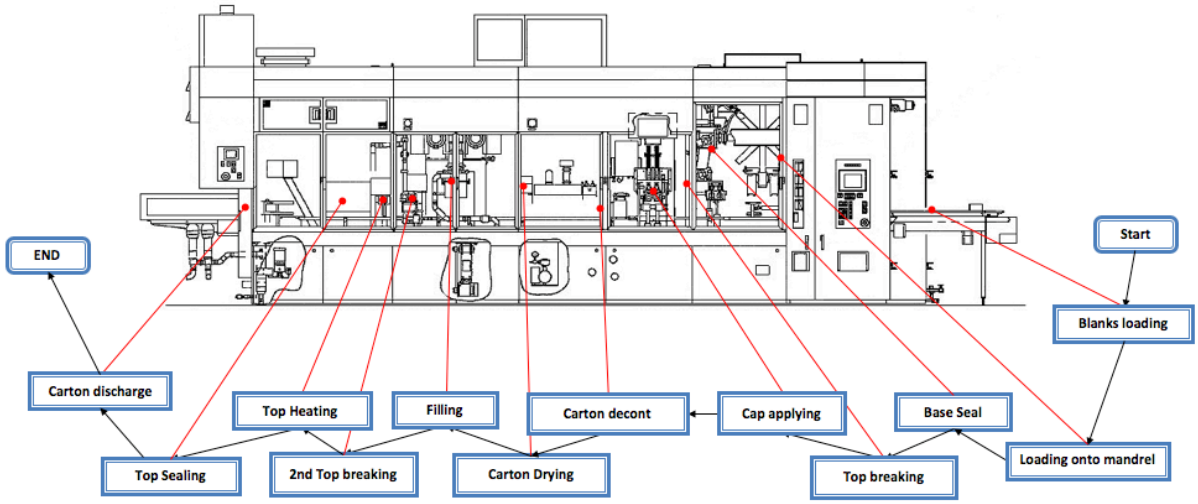

Figure 3: Illustration of processing steps for filling product in a filling machine for gable top cartons. Operating sections described.

1.3 GAS BARRIER IMPERFECTIONS

A perforation formed in the polymer layers in a carton during production or filling is not desired as it can increase the amount of oxygen to permeate the package and thereby have the potential to decrease the quality of the orange juice. Perforations in gable top cartons usually occurs in one of two ways. Either in the crease lines caused by stress in the folding process, or by over- or underuse of heat in one of the welding processes. Both can create barrier imperfections that are highly irregular in shape and size that can be difficult to locate on the board. The variance in size, shape and placement of barrier imperfections affect the permeation of oxygen (Larsen & Liland, 2013, Allan-Wojtas et al. 2008). For explaining differences in gas permeation in varying packages it is necessary to describe the microstructure and geometric features of the barrier perforations in the material. (Larsen, Liland, 2013, Disimile et al. 1998).

The most common tool in the industry to investigate carton integrity in a production facility, is with the use of the dye-test method. A dye-test exposes the finished package to a solvent with a coloring agent added to reveal possible perforations. A dye penetrant solution is either poured into a part of a finished carton or applied to a carton surface. After contact with dye penetrant for a minimum specified time the carton will be rinsed with water and the inside and outside of the carton is inspected visually for dye spots on the board. If perforations are discovered the carton could be further inspected in a microscope to attempt to clarify the cause of the formation of the perforation.

In a production facility, the situation usually demands rapid responses if imperfections appear. If perforations are caused by a filling machine, operating personnel can change filler heater and mechanical settings until no dye spots are present in a specified selection of cartons. Which machine settings to change and why are usually dependent on experience in the operators of the filling machines (Own observations).

The irregularity of a barrier imperfection in a gable top carton can be caused by the way the carton is constructed. In a carton with several layers of polymers with different melting points the combination of physical stress can form barrier imperfections differently in the layers and be extremely difficult to locate. Thus, the cause of and shape of a barrier imperfection could also be difficult to determine (Larsen & Liland, 2013).

1.4 VITAMIN C IN ORANGE JUICE

Vitamin C is an essential nutrient and consumption is related to several health benefits with lowering the risk of cancers, cardiovascular diseases and aging. Consumption of vitamin C also prevents scurvy, a deadly disease caused by the lack of vitamin C in the diet. This is a condition that is no longer common in the developed world, but can still be prevalent in third world populations (Gabriel et al. 2015, Plaza et al. 2005). Vitamin C functions as an antioxidant by directly scavenging singlet oxygen, hydrogen peroxide and hydroxyl radicals. Women and men need 75 and 90 mg vitamin C each day, respectively, which is easily consumed with a balanced western diet. A very common source of Vitamin C in the diet of an average adult is fruit juices, with the most common being orange juice. A glass of orange juice can, depending of Vitamin C-content, provide 30-80% of the recommended daily intake (Klimczak et al. 2006).

With consumers relying on orange juice to cover their regular vitamin C intake it is essential that the orange juice sold in stores, even close to the expiration date, contains as much vitamin C as is claimed by the manufacturer of the product. The regulations concerning the labelling of nutritional values of food in Norwegian markets are decided by the EU commission of Health and Consumers Directorate. EU regulation 1169/2011, state that for goods that contain more than 7,5% of the required daily intake of a nutritional compound in a drinkable product, must label this information on the package. The regulation applying to vitamin C states that the labelling must show never more than 35% under and 50% over and the actual value in the product for the entire shelf life. Although an exception has been made for vitamin C in drinkable products, where it is accepted to exceed the +50% limit. These limits include measuring uncertainty. For example, regulation (EU) No 1169/2011 then allows for a minimum level of 19,5 mg/100 ml orange juice during the entire shelf life if the carton is branded with 30 mg/100 ml on the label. The regulation allows for rounding of the values when measuring with two decimals (Regulation (EU) No 1169/2011).

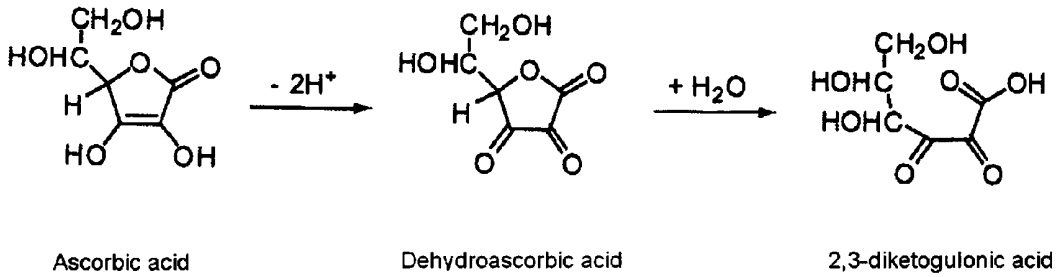

Figure 4: Ascorbic acid (vitamin C) oxidation route in a liquid.

Oxygen from air incorporated into orange juice that occurs during preparation, filling and transport are the reasons for vitamin C loss. In addition to dissolved oxygen in the orange juice there is an amount of oxygen in the headspace of the carton, that contributes to vitamin C deterioration over time. The pathway for degradation of vitamin C in the presence of oxygen is illustrated in Figure 4. Dehydroascorbic acid (DHA) and diketugulonic acid (DKA) are formed as products during oxidation of vitamin C in a liquid. DHA contributes to antiascorbic activity, unlike DKA, resulting in the total antiascorbic activity in juice being a combination of AA- and DHA-content. Where DHA contributes a share of ≈1-2% (Zerdin et al. 2002) (Nagy 1980).

1.5 AOIR

Transmission rate is the measurement of the quantity of gas that passes through a material with a known area over a given time. The rate of gas flow is determined by temperature and the gas concentration difference between the atmosphere and the headspace in the package. In perforated packages tested in this study the total OTR will be the sum of gas movement through perforations and the polymeric barriers.

The AOIR method (Larsen 2000) is based on measuring the O_2 concentration in the headspace of a package two times with a given interval and let the PermMate-system calculate the OTR of the package based on measurement interval, time of measurements and package volume. This method has been proven to work for packages with low permeability (0.06 to 1.4 ml O_2/pack/day), but no publications validate the method on packages with higher OTR levels. AOIR as developed by Hanne Larsen and made available for use in the PermMate-system made by PBI Dansensor was to be investigated as a possible method for determining the OTR of gable top cartons. The AOIR method was chosen because of the major limitations of the OxTran-system widely used today. OxTran has an upper sensitivity limit of 5 ml O_2/package/day per the user manual for Mocon OxTran 2/61. Although the upper limit of detection for the OxTran has been measured to 1,5 ml O_2/package/day in laboratory tests at Elopak (Sara Lisboa, Elopak, 8.12.16), which is too low for a package with perforations. The AOIR method also allows for testing samples at low temperatures, while OxTran has a lower temperature limit of 20°C (http://www.mocon.com/assets/documents/oxtran261.pdf/ 1.12.16). In 2000 the AOIR-method was proven to work in comparison to OxTran with a repeatability of 2.6% for packages with low permeability (Larsen 2000).

The transfer rates of different gases vary for perforated and non-perforated films. In non-perforated films, CO_2 will travel 2 to 8 times faster than O_2 through the film (Allan-Wojtas et al. 2008*)*. Allan-Wojtas et al. (2008) also found that microperforations with a diameter between 30 µm and 100 µm had a gas transfer rate directly proportional to the area of the perforation under calm conditions. They also found that for >55 µm diameter of the perforation the diffusion rate was no longer predictable due to convection.
Larsen and Liland (2013) found no increase in permeability in packages with 75-90 µm diameter perforations when increasing temperature from 5°C to 23°C. While in packages with no perforations the O_2 permeability increased by a factor of 2.4.

The basis of OTR calculation in the AOIR-method is the following equation:

$$TR = -\frac{V}{t_f - t_i} \ln\left(\frac{C_{air} - C_f}{C_{air} - C_i}\right) \quad (1)$$

V is the volume of the package tested, , t_f is the time of final gas concentration measurement t_i the time of the first gas concentration measurement, C_{air} the concentration of gas in the ambient atmosphere, C_f is the concentration of gas in the package at the final measurement, C_i is the concentration of gas in the package at the first measurement. C_{air} 0.21 O_2 (Larsen & Liland 2013).

The volume increase effect is caused by O_2 permeating faster through a membrane than N_2 at a relation of 4:1. This will result in O_2 entering a carton package faster than N_2 can permeate out of the package and increase the volume and/or pressure in the carton (Moyls 2004).

2. MATERIALS AND METHODS

2.1 CREATION OF SEAL IMPERFECTIONS

The irregularity of naturally formed damage to gas barriers complicated the task of creating perforations with dimensions and properties that were comparable to perforations formed in production. The perforations or barrier imperfections produced in the carton blanks originated from two different methods.

1. Manually perforating the gas barrier of the carton from the inside with a thin needle fixed with a butterfly-bolt in an in-house made cylinder. The length of the needle tip to perforate the material could be adjusted by increments of 100 µm with separator rings on top of a steel plate. Perforation tool pictured in Figure 5. When perforating gas barriers in the

cartons that were investigated in this thesis, three separator rings were used to get a theoretical total penetration depth of 300 µm. This was to achieve a hole diameter of 100 µm, as the needle was cylindrical. The needle was a common sewing needle. The 100 µm barrier imperfections were only applied to EVOH cartons and 5 barrier imperfections were stamped in each carton blank.

The 500 µm perforation were made with another tool made in-house at Elopak. A metal rod with a marginally larger than 500 µm diameter had been drilled and hollowed out to an inner diameter of 500 µm. The cylinder could then be used as a stamping tool to lift out the material cut out in the center. Perforation tool pictured in Figure 5. 500 µm perforations were created in EVOH-barrier and aluminum-barrier cartons.

Figure 5: Tools used to manually perforate gas barrier in EVOH- and aluminum-barrier gable top cartons. From the left steel plate with needle perforation depth adjustment rings, cylinder for holding needle, stamping tool for moving needle in the cylinder and tool for creating 500 µm perforations. Made in workshop at Elopak, Spikkestad.

2. The second method for producing barrier imperfections in the carton material was through the use of too much or too little heat in the standard process for preparing a blank in the Elopak blank factory or in the filling machine. In the process of creating a blank from the flat-blank, as previously described, the 1. panel is welded to the 5. panel by heating the PE polymer. This process was performed in a manually controlled converting machine and the temperature was increased until barrier imperfections were visible along the 1. panel or the 5. panel after a dye-test. Along the first panel the heat was adjusted until approximately 10 barrier imperfections appeared along the 1. panel above the top major horizontal crease line. Then heat was adjusted further until a continuous row of barrier imperfections appeared along the 1. panel. Lastly/finally the heater for the 5. panel welding was adjusted until

approximately 10 perforations appeared along the 5. panel above the top major horizontal crease line. During filling the top-sealer heater was adjusted to over-heat until one or more barrier imperfections appeared on the gable top seal area with a 10-minute dye test. Furthermore, heat was lowered until a complete weld was not achieved and a dye-test indicated imperfections in gas barrier under the gable top seal. These heating errors were all created on EVOH-cartons. A complete overview of the cartons with barrier imperfections and reference cartons with no imperfections are presented in Table 1.

Table 1: Sample numbering and gas barrier imperfections investigated in the thesis. EVOH- and aluminium-barrier cartons were 1 liter. Barrier imperfections along 1. and 5. panels as well as overheated and underheated gable tops were machine made. Perforations with diameter 100 µm and 500µm were manually made.

Sample number	Gas barrier	Failures
1	EVOH	Reference with no barrier imperfections.
2	EVOH	5 barrier imperfections, 100 µm.
3	EVOH	1 barrier imperfection, 500 µm.
4	EVOH	Barrier imperfections along the 1. panel.
5	EVOH	Continuous barrier imperfections along the 1. panel.
6	EVOH	Barrier imperfections along the 5. panel.
7	EVOH	Over-heated gable top.
8	EVOH	Under-heated gable top.
9	Aluminum	Reference with no barrier imperfections.
10	Aluminum	1 barrier imperfection, 500 µm.

2.2 FILLING MACHINE SETTINGS

In the days leading up to filling of product in cartons, the filling machine, S-PSF65UC, Shikoku, Japan was tested with carton blanks to be used in the experiment. Testing was performed to find optimal temperature settings in filling machine for the references of the different materials and the temperature for the over-heated and the under-heated gable tops in the EVOH barrier cartons. The temperatures used are presented in Table 2.

Table 2: Carton top heater settings in filling machine for EVOH- and aluminum gas barrier cartons. Filler 1 is top sealer heater and filler 2 is top sealer pre-heater.

Heater settings	Temperature 1	Temperature 2
Reference EVOH	300°C	300°C
Under-heated gable top EVOH	270°C	220°C
Over-heated gable top EVOH	450°C	400°C
Aluminum	390°C	390°C

2.3 ORANGE JUICE

The juice was prepared from concentrate packed and delivered by Medibel in Holland and produced from of juice from Brazil. The specifications of the juice tested at Medibel are specified in table in attachment 4.

2.4 PROCESSING AND FILLING

Orange juice concentrate was thawed for four days in a refrigerated room at 8°C. The concentrate was then pumped into a mixing tank and diluted with cold water until a final sugar content of 11.3 °Bx. At this point the total volume was 1350 liters made from 250 kg of concentrate. The mixed juice was pasteurized in a tubular heat exchanger at 93°C for 30 seconds. Filling was performed at 13-17°C. The density was measured to 1041g/1000 ml. Each series of 60 cartons were filled in runs of 10 cartons and placed manually on a rolling container. Filling order and filler settings are presented in table 3. A separate automatic printer (Domino A300, Domino Amjet inc., Illinois, USA) was placed after the filling machine and programmed to mark all cartons with a sample number. Filling order instruction used by filling machine operator presented in Table 3.

Table3: Filling order and filler temperature settings for all EVOH- and aluminum-barrier cartons used on day of filling by operating personnel.

Order	**Carton**	**Comments**	**Amount**	**H 1**	**H 2**
1	EVOH	Reference	60	**300°C**	**300°C**
2	EVOH	5 x 100 µm	60	300°C	300°C
3	EVOH	1 X 500 µm	60	300°C	300°C
4	EVOH	Perforations 1. panel	60	300°C	300°C
5	EVOH	Continuous 1. panel	60	300°C	300°C
6	EVOH	Perforations 5. panel	60	300°C	300°C
7	EVOH	Under-heated top	60	**270°C**	**220°C**
8	Aluminum	Reference	60	**390°C**	**390°C**
9	EVOH	Over-heated top	60	**450°C**	**400°C**
10	Aluminum	1 x 500 µm	60	390°C	390°C

2.5 INCUBATION

Immediately after filling, cartons were placed in a dark refrigerated room set to 8°C in standard rolling containers. A temperature logger (Testo 176 T4, Testo, Germany) was placed with the samples as well as two temperature loggers (Steril Disk Probe, Technosoft, Italy) placed inside the product in two separate cartons in separate locations in the storage room. Temperature loggers were set at a 15-minute recording interval.

Samples were taken out for testing six times during storage. At 28.09.16, 05.10.16, 21.10.16, 02.11.16, 16.11.16 and 30.11.16. At the dates of outtake dissolved oxygen content, headspace oxygen concentration in carton and Vitamin C content in the juice was tested. AOIR preparation and dye-test of gable tops was performed the days following.

2.6 CARTON HEADSPACE OXYGEN CONCENTRATION

Headspace oxygen concentration (%) was analyzed at Elopak according to Elopak Company Method ECM10201 *Headspace gas analysis of CO_2 and O_2 using a Micro Gas Chromatograph,* with some modifications (Attachment 2 in appendix**).**

The oxygen concentration in the headspace was analyzed with a CheckMate II O_2 and CO_2 analyzer built by PBI-Dansensor A/S in Ringsted, Denmark. The CheckMate II instrument has a zirconia-based O_2-sensor and a Non-Dispersive Infrared CO_2 sensor. The process was adapted to each carton series, depending of the type of error on the carton gable-top. In sample series 1, 4, 5, 6, 7, 8, 9 and 10 the board was removed on the first panel, above the top major horizontal crease line. In series 2 and 3 with manual made barrier imperfections on the first panel the septum was placed on the cap and later fixed with epoxy glue. Aluminum-barrier cartons used where of the traditional variety with no screw-top closure, which allowed for removal of board on the gable top without interfering with perforations. Board removal procedure is described in chapter 2.9.2. After attachment of septum on either EVOH-barrier or aluminum-barrier after board removal or on the cap, a syringe needle with 0,8 mm diameter was inserted through the septum and the PermMate software was used to read O_2 level with the CheckMate II.

The zirconia sensor in the CheckMate II system, for measuring O_2 concentration, can measure with a resolution of 0,1% in the range above 10% with an absolute accuracy of ±1% relative in the range over 1% (Larsen, 2000).

2.7 DISSOLVED OXYGEN CONTENT IN JUICE

The dissolved oxygen content was analyzed at Elopak per Elopak Company Method ECM10209 *Dissolved oxygen analysis in water and fruit juices by using a Micro O_2 Logger,* with some modifications to the process (Attachment in 1 appendix).

After headspace oxygen was measured and logged the gable top was cut approximately 5 mm under the upper horizontal creasing line from the first panel and around the carton. Cutting was performed with a sharp snap-off blade kept in water when not in use to prevent buildup of orange juice and corrosion to dull the blade. The Mettler Toledo Seven Go Pro SG9 (Schwerzenbach, Switzerland) was calibrated with deionized water and placed immediately in the juice carton after cutting. The sensor was placed approximately 7 cm under the juice surface. The sensor was moved to a new sample as soon as the previous reading was done. The results were noted by operator.

At week 8, all samples were tested two times. One measurement performed as described, with no mixing of the juice. In addition, one measurement was performed after carefully mixing the juice with a glass rod until all sediment from the bottom of the juice was distributed evenly.

2.8 VITAMIN C CONTENT IN JUICE

The content of vitamin C was analyzed at Elopak per Elopak Company Method ECM10212 *Vitamin C in juices (2,6-dichlorophenol-indophenol titrimetric method* (Attachment 2. in appendix). The method is based on Vitamin C reducing oxidation-reduction indicator dye, 2,6-diclorphenol-indophenol, to a colorless solution. At the endpoint, the excess unreduced dye was rose pink in an acid solution.

The equipment used for titration was:

- 716 DMS Titrino, Methrom, Herisau Switzerland.
- 730 sample changer, with 15 slots, Methrom, Herisau Switzerland.
- A redox electrode, Methrom, 6.0451.100, Herisau Switzerland.
- Tiamo 2.5 software for Windows, Methrom, Herisau Switzerland.
- PH meter, PHM92, Radiometer, Copenhagen, Denmark
- Balance, Mettler Toledo, AX105, DeltaRange, Schwerzenbach, Switzerland.
- Magnetic stirrer with heater.
- 8 liters HPO_3 and potassium hydroxide buffer at pH 3,5-4, Merck, Germany.

- 2,6-dichlorphenolindophenol Na-salt dihydrate standard solution, Merck, Germany.
- 50 mg/100 ml solution of ascorbic acid in buffer.

The juice sample was titrated in the presence of meta-phosphoric acid solution to prevent auto-oxidation of the ascorbic acid at high pH. One day before vitamin C analysis, 8 liters of buffer solution and 500 ml Indophenol standard solution was prepared. This modification to the process was chosen to conserve time, as to be able to perform vitamin C analysis for up to 50 samples in one day. On the day of testing the juice was transferred to a 1000 ml beaker for mixing the content of the carton thoroughly to measure a correct average of the vitamin C content in the orange juice for the entire carton. This was not done at week 1 and 2. 10 ml sample was transferred to a 150 ml beaker with a 10 ml full pipette. Immediately, 120 ml of buffer was added to prevent oxidation in the sample.

When the standard solution was made the day before analysis the flask was kept wrapped in aluminum foil to avoid light exposure. Vitamin C standard solution was made the same day as the analysis. Testing was performed in accordance with standard procedure in the Tiamo software. Tiamo 2.5 software calculated the vitamin C content automatically and stored the data. Mg ascorbic acid/ 100 ml juice = T * EP1 *10, where T = factor from standardization and EP1 = ml indophenol standard solution used.

A test was performed at week 4 to investigate if there was a difference in vitamin C content in the bottom and the top of the juice in one carton. One sample was taken from the bottom of a carton, one from the top and one after mixing the juice. The results, described in chapter 3.3, prompted a decision to retest all samples at outtake week 4. This caused a two day delay of testing at week 4.

2.9 AOIR

The AOIR solution from PBI Dansensor is comprised of a CheckMate II system combined with a PermMate (PBI-Dansensor, Ringsted, Denmark) system combined with the PermMate 2.0 software (PBI-Dansensor, Ringsted, Denmark) on Windows XP. The method utilized flushing of the package with Nitrogen and measure change in oxygen concentrations over time to calculate OTR. Precut gable tops were fixed to a metal foil with epoxy glue. The metal foil was taped to steel plates too keep the foil flat and prevent the glue from becoming unfastened. Epoxy glue was applied to the edge of the board to prevent gas seepage through the carton fiber raw edge. The foil had previously been measured to have an oxygen transmission rate below 0,05 ml/pack/day, with an OxTran-system at Elopak (Sara Lisboa,

Elopak, 15.11.16). Instructions in the PermMate 2.0 software were followed and the gable tops flushed to a concentration 5% O_2. The gable tops were set in a refrigerator at 8°C for the duration of storage. Calculation of OTR with the AOIR-method demanded measuring oxygen concentration two times, with a minimum increase in oxygen concentration in the package to ensure accuracy. Minimum oxygen concentration increase in a package between measurements described in Table 4.

Table 4: Minimum increase in oxygen concentration in a package between measurements for use of the AOIR-method with PermMate II, described in PBI-Dansensor manual.

OTR	Minimum increase
Low OTR: <0.01 ml O_2/pack/day	>0.05%
Medium OTR: 0.01-0.1 ml O_2/pack/day	>0.03%
High OTR: >0.1 ml O_2/pack/day	>0.1%

2.9.1 VOLUME OF GABLE TOPS

The AOIR-method demands that the volume (ml) of packages for testing are known. The gable tops were cut manually of the carton with a break-of knife and therefore subject to some variation in volume. It was decided to determine the volume of a selection of gable tops and in further investigations use the average volume in the calculations of the OTR. Volumes were determined with the PermMate-system.

2.9.2 GABLE TOP PREPARATION FOR AOIR

In gable tops from series 1, 4, 5, 6, 7, 8, 9 and 10 a portion of the outer board and PE was removed to place septum directly in the gas barrier, as shown in Figure 6. Septum placement on the closure was used as an alternative placement option in gable tops from cartons in series 2 and 3. Board and PE removal was accomplished by cutting a square in the outer PE-layer with a sharp blade and tearing of the PE, adding water to the exposed board to soften fiber and scraping the board until all fiber was removed. The gas barrier of the carton would be preserved as the EVOH barrier was protected against the water with a layer of PE.

Figure 6: Exposure of EVOH gas barrier after removal of outer PE and board layer on gable top carton for allowing septum placement directly on gas barrier.

2.10 Dye fluid test

After completion of AOIR measurements the gable tops were removed from the foil. The gable tops were placed upside-down on a rack and filled to approximately 50% of total volume with an ethanol solution with E131 Patentblue. After 10 minutes the gable tops were rinsed with water and inspected for dye spots on the inside and the outside. The area (mm^2) of individual dye spots was measured and logged. Images of representative dye spots for each of the generated barrier imperfections are presented in figure 7.

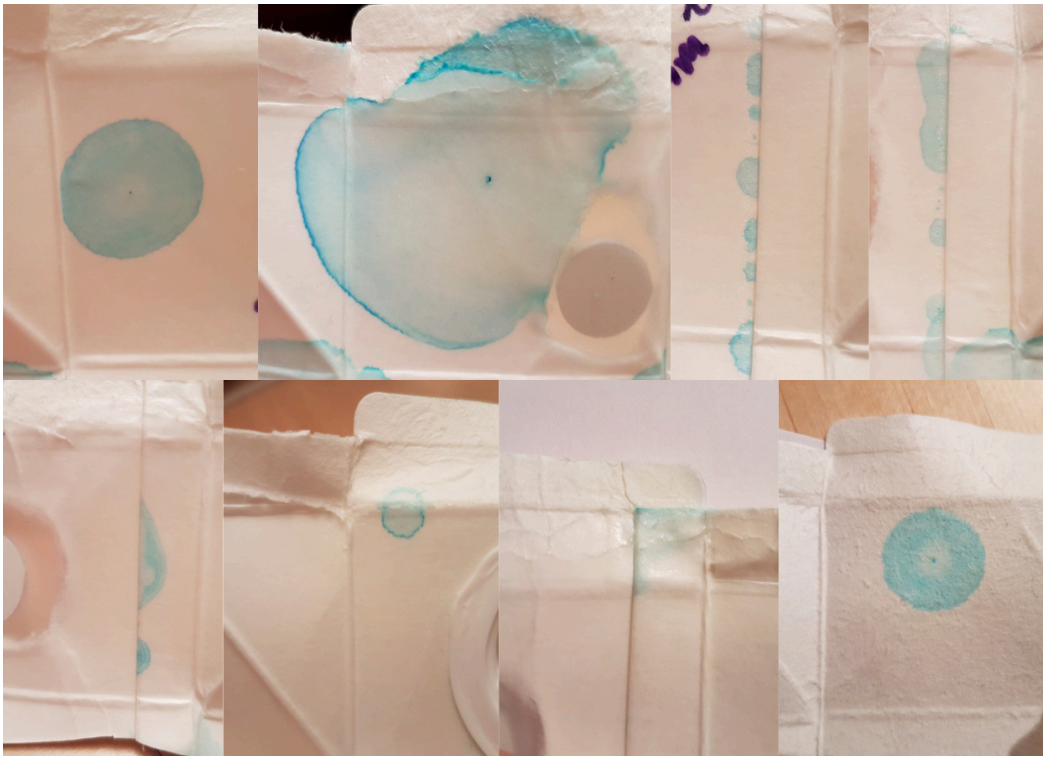

Figure 7: A representation of dye spot penetration in gable top cartons with EVOH and aluminum gas barrier perforations tested in samples 2, 3, 4, 5, 6, 7, 8 and 10, from top left to bottom right.

2.11 Microscopy

The parts of the board with manual made perforations were cut out from the gable top, the board layers were separated and submerged in regular soap for 30 minutes. The board and polymer was then placed in 60% NaOH for 30 minutes to dissolve the board. The samples were rinsed and dried before perforations were photographed in a Nikon SMZ 1270 stereo microscope (Nikon, Japan).

2.13 Sensory analysis

A sensory analysis was performed at week 8 to test if a panel was able to detect a difference in taste or smell in samples with a low and high degree of vitamin C deterioration in orange juice. Two triangle tests were set up with a 10-person panel. Samples were tested under red light to prevent panel inspecting color. Samples were presented with 3-digit sample codes and were randomized in presentation order. As test 1. was completed the panelist was served test 2. Samples were served at 12°C. Previous tests at Elopak experienced a limit of detection of difference in a triangle test, at a vitamin C content difference of 10 mg/100 ml (Liv Bente Strandos, Elopak, 5.10.16).

1. Sample 9 (Aluminum reference) (x 2) and sample 4 (x 1) (barrier imperfections along the 1. panel).
2. Sample 1 (EVOH reference) (x 2) and sample 4 (x 1) (barrier imperfections along the 1. panel).

2.12 Statistical analysis

The software used for statistical analysis was MINITAB 7 provided by NMBU, Ås.

A) A Tukey test for statistical difference was performed for headspace oxygen concentration, dissolved oxygen concentration and vitamin C content. The test provided letters, where samples described with the same letter were not significantly different.

B) A balanced ANOVA was conducted to examine the effect of responses and main effects BI (Barrier Imperfection), WN (Storage time) and interaction BI*WN in EVOH cartons with barrier imperfections.

C) A balanced ANOVA test was performed for the effect of responses and main effects BI (Barrier Imperfection), WN (Storage time) and interaction BI*WN in EVOH an aluminum barrier cartons with no perforations

D) A balanced ANOVA test was performed for the effect of responses and main effects BI (Barrier Imperfection), WN (Storage time) and interaction BI*WN in EVOH an aluminum barrier cartons with 500 μm perforations.
C) An analysis of correlation was performed for all samples to reveal possible correlation between Vitamin C/Headspace O_2, Vitamin C/Dissolved O_2, and dissolved O_2/Headspace O_2.
D) Correlation tests were performed for dissolved oxygen content in orange juice measured with and without mixing and vitamin C content in orange juice at week 8.
C) An analysis of correlation was performed for revealing possible correlation between dye test area (mm^2) and AOIR, dye test area (mm^2) and vitamin C content as well as AOIR and vitamin C content.
E) A Tukey test for statistical difference was performed for AOIR measurements. The test provided letters, where samples described with the same letter were not significantly different.

3. RESULTS

3.1 TEMPERATURE LOGGERS

Table 5: Temperature averages with standard deviations for temperature loggers in cooling room and logger 1 and 2 in products. 8 weeks of storage, measurement every 15. minute.

Placement of logger	Average temperature with SD
Cooling chamber	7,78 ± 0,64°C
Product	8,25 ± 0,28°C
Product	7,52 ± 0,31°C

Temperature recorded in the cooling chamber, presented in Table 5, was close to the 8°C set temperature at 7,78 ± 0,64°C. Temperature recorded in product 1 and 2 had a difference of 0,73°C at 8,25 ± 0,28°C and 7,52 ± 0,31°C.

3.2 Headspace Oxygen concentration

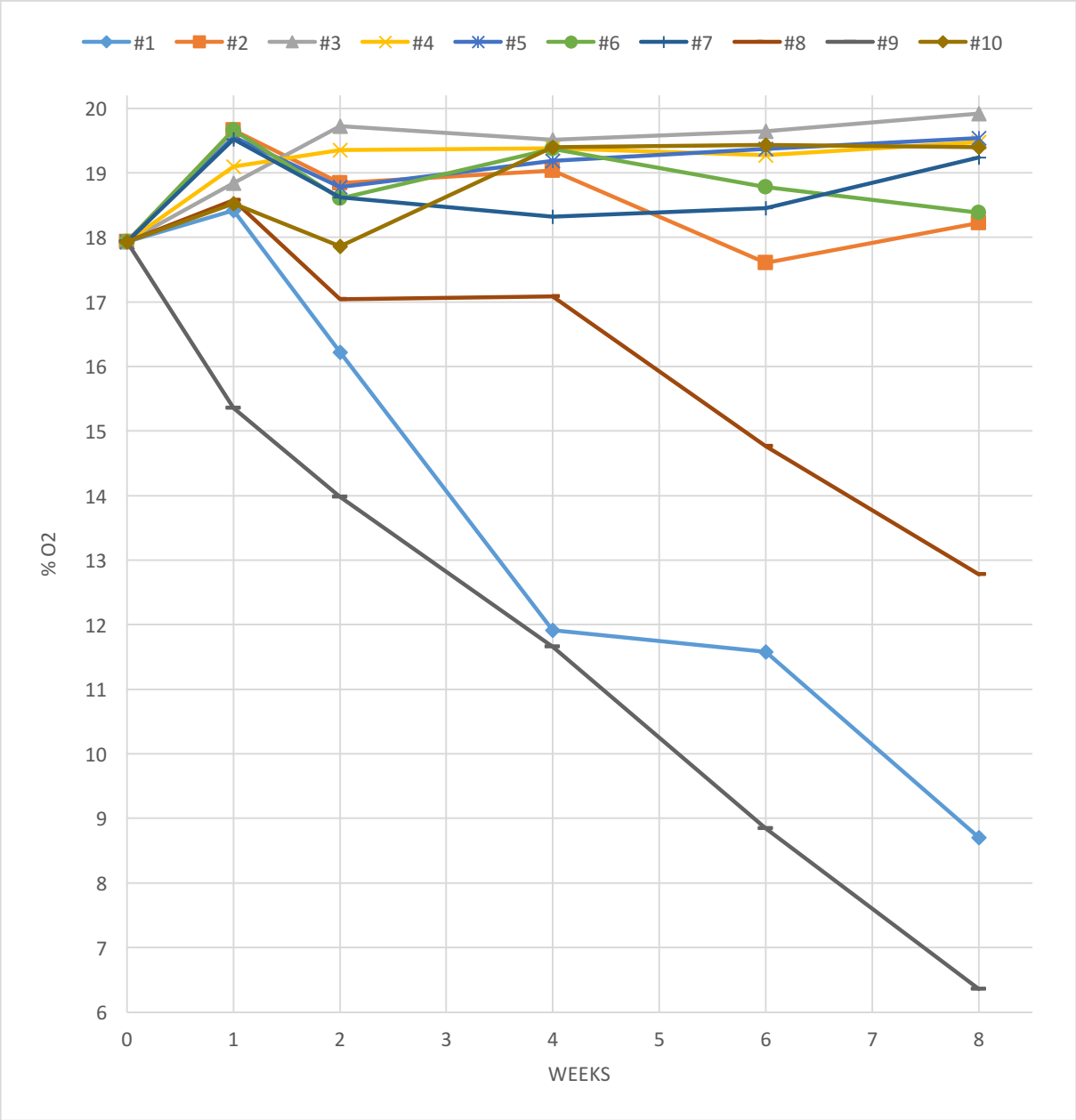

Figure 8: Headspace oxygen concentration development in gable top cartons with and without barrier imperfections over the duration of 8 weeks. Each parallel represents an average of 5 cartons tested.

Development in gable top headspace oxygen concentration is presented in Figure 8, showing a reduction in sample 1, 8 and 9. All other samples retained a concentration of oxygen over 18% in the gable top, except for sample 10 at week 2 and sample 2 at week 6. Table 6 contains numeric values for oxygen concentration measured over 8 weeks, with standard deviation. Letters in Table 6 represents statistical significant difference (Tukey test, CI = 95%), where samples presented with the same letter are not different.

Table 6: Headspace oxygen concentration in gable top cartons with and without barrier imperfections, with standard deviation. Samples tested over 8 weeks, each value represents an average of 5 cartons tested. Letters represent statistical significant difference tested with a Tukey-test at 95% significance. Samples presented with the same letter are not different

Sample	Start	Week 1	Week 2	Week 4	Week 6	Week 8
#1	17,93±0,21	18,42±0,44 B	16,22±1,07 BC	11,91±0,95 C	11,58±0,94 C	8,70±0,67 C
#2	17,93±0,21	19,66±0,06 A	18,84±1,27 AB	19,03±0,30 A	17,60±3,80 AB	18,22±3,53 A
#3	17,93±0,21	18,84±1,27 AB	19,72±0,04 A	19,51±0,04 A	19,64±0,04 A	19,92±0,03 A
#4	17,93±0,21	19,10±0,82 AB	19,35±0,24 AB	19,38±0,47 A	19,27±0,20 A	19,48±0,41 A
#5	17,93±0,21	19,56±0,29 AB	18,78±1,46 AB	19,18±0,32 A	19,37±0,21 A	19,54±0,27 A
#6	17,93±0,21	19,66±0,09 A	18,60±1,53 AB	19,37±0,14 A	18,78±0,39 A	18,38±1,56 A
#7	17,93±0,21	19,52±0,27 AB	18,62±0,86 AB	18,32±1,34 AB	18,46±1,73 A	19,24±0,43 A
#8	17,93±0,21	18,58±0,23 AB	17,04±0,52 AB	17,09±0,91 B	14,77±0,63 B	12,78±1,32 B
#9	17,93±0,21	15,36±0,34 C	13,98±0,22 C	11,66±0,20 C	8,85±0,37 C	6,36±0,34 C
#10	17,93±0,21	18,52±0,30 AB	17,86±2,30 AB	19,4±0,05 A	19,43±0,05 A	19,4±1,00 A

3.3 Dissolved Oxygen concentration

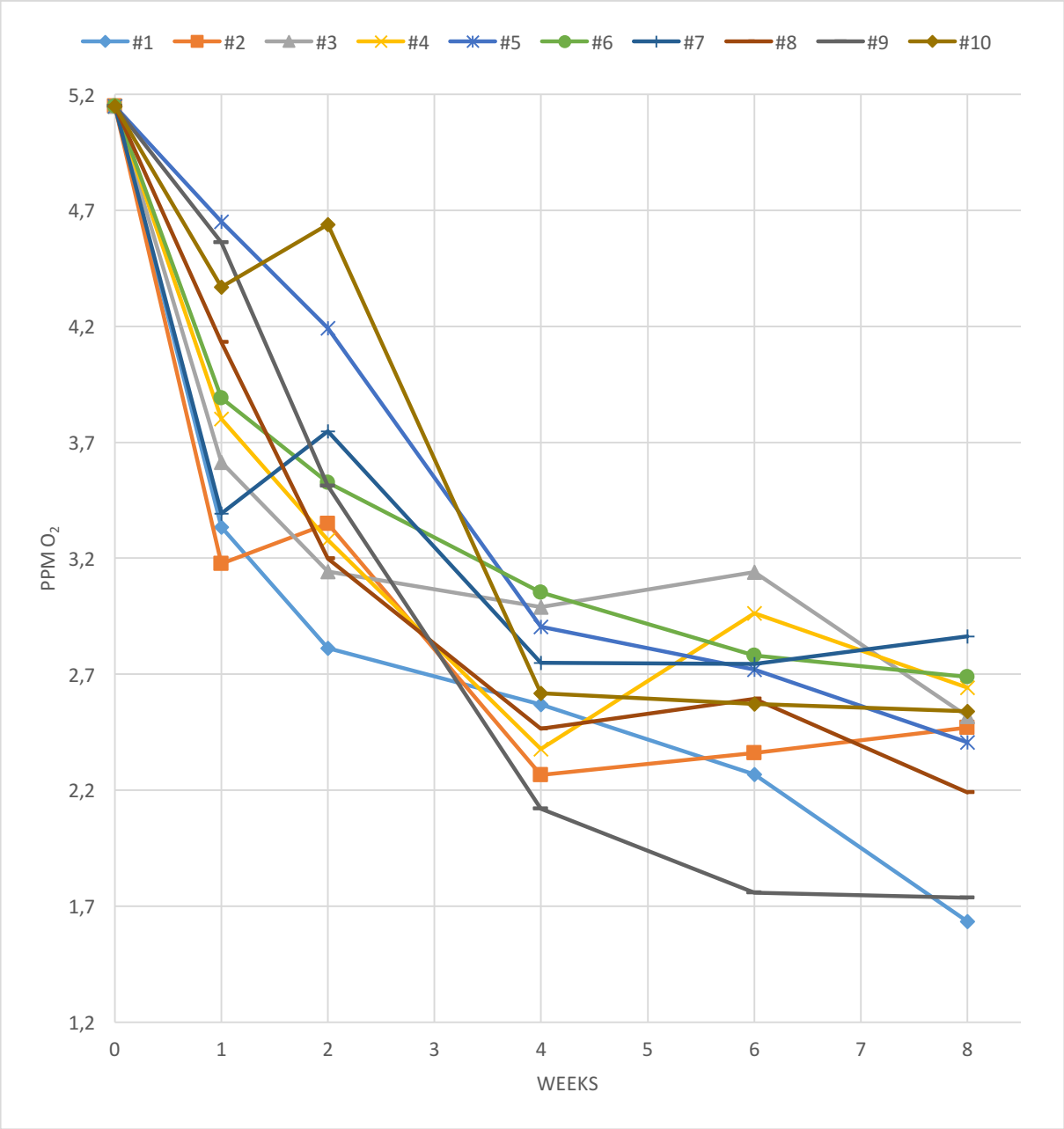

Figure 9: Dissolved oxygen content development in juice stored in gable top cartons with and without barrier imperfections over the duration of 8 weeks. Each parallel represents an average dissolved oxygen content in 5 cartons tested.

Development in orange juice dissolved oxygen content is presented in Figure 9. All samples displayed a reduction in dissolved oxygen content, with the lowest content found in sample 1 and 9 at 8 weeks of storage. Table 7 contains numeric values for dissolved oxygen content measured over 8 weeks, with standard deviation. Letters in Table 7 represents statistical significant difference (Tukey test, CI = 95%), where samples presented with the same letter are not different.

Table 7: Dissolved oxygen in gable top cartons with and without barrier imperfections, with standard deviation. Samples tested over 8 weeks, each value represents an average of 5 cartons tested. Letters represent statistical significant difference tested with a Tukey-test at 95% significance. Samples presented with the same letter are not different

Sample	Start	Week 1	Week 2	Week 4	Week 6	Week 8
		3,33±0,05	2,81±0,17	2,57±0,36	2,27±0,31	1,63±0,17
#1	5,15±0,18	BC	D	ABCDE	CD	D
		3,18±0,80	3,35±0,55	2,27±0,19	2,36±0,35	2,47±0,30
#2	5,15±0,18	C	BCD	DE	C	AB
		3,61±0.77	3,14±0,50	2,99±0,21	3,14±0,15	2,52±0,24
#3	5,15±0,18	ABC	CD	AB	A	AB
		3,80±0,54	3,28±0,55	2,38±0,11	2,96±0,29	2,64±0,12
#4	5,15±0,18	ABC	CD	CDE	AB	AB
		4,65±0,27	4,19±0,60	2,90±0,37	2,72±0,24	2,41±0,15
#5	5,15±0,18	A	AB	ABC	ABC	AB
		3,89±0,23	3,53±0,23	3,05±0,21	2,78±0,16	2,69±0,14
#6	5,15±0,18	ABC	BCD	A	ABC	AB
		3,39±0,60	3,75±0,16	2,75±0,21	2,74±0,16	2,86±0,18
#7	5,15±0,18	BC	BC	ABCD	ABC	A
		4,13±0,16	3,20±0,44	2,47±0,44	2,59±0,40	2,19±0,33
#8	5,15±0,18	ABC	CD	BCDE	ABC	BC
		4,56±0,59	3,51±0,12	2,12±0,19	1,76±0,17	1,74±0,35
#9	5,15±0,18	A	BCD	E	D	CD
		4,37±0,22	4,64±0,28	2,62±0,17	2,57±0,28	2,54±0,26
#10	5,15±0,18	AB	A	ABCDE	BC	AB

3.3.1 Dissolved oxygen with and without mixing of juice in carton

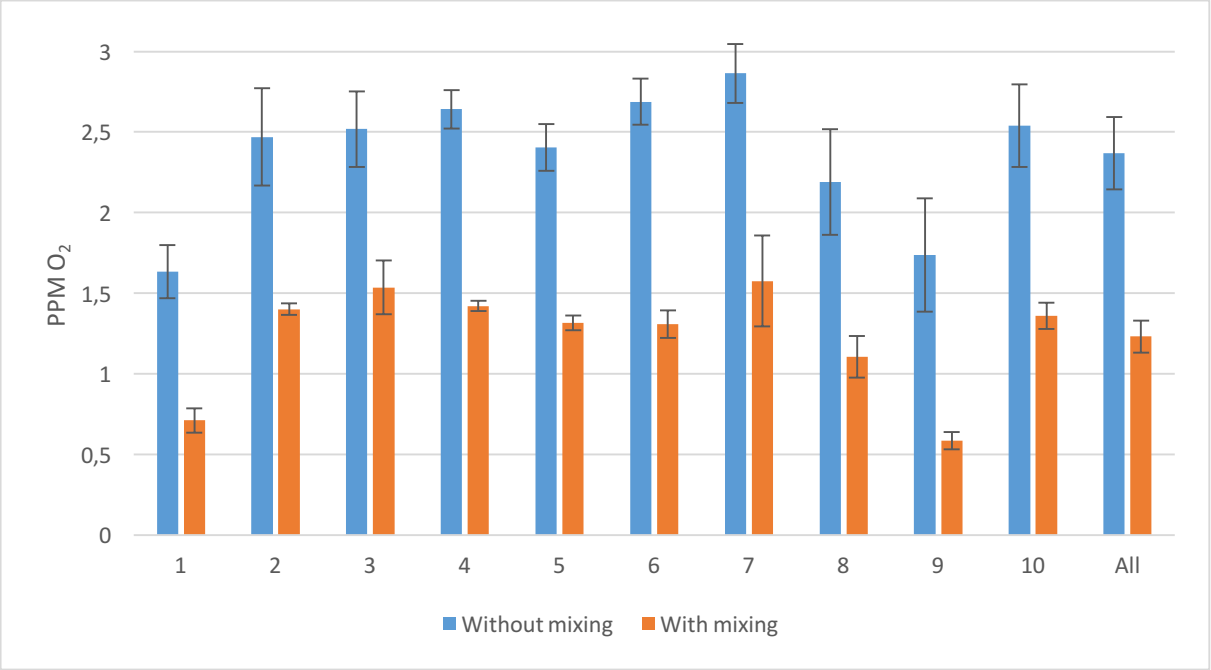

Figure 10: Dissolved oxygen content measured in juice stored in gable top cartons with and without barrier imperfections. Each value represents an average of 5 cartons tested, and the far right an average of all 50 samples, with standard deviation. Measurement performed before (blue) and after (orange) mixing of the same juice in each carton.

Mixing the juice in the samples caused a decrease in the measured dissolved oxygen content in all cartons. Measured average value for 50 cartons displayed a decrease in dissolved oxygen content of 48% and a decrease in standard deviation of 56%. Figure 10 shows results of dissolved oxygen measurement in 50 juice cartons before and after mixing.

Table 8: Correlation test, dissolved oxygen content measurement before and after mixing orange juice, stored in gable top carton for 8 weeks and vitamin C-content measured in the same 50 samples.

	Dissolved O_2 without mixing juice	Dissolved O_2 with mixing juice
Dissolved O_2 with mixing juice	C: 0,827 P: 0,000	
Vitamin C-content	C: -0,478 P: 0,000	C: -0,454 P: 0,001

A correlation test between dissolved oxygen content measurement before and after mixing the orange juice, and vitamin C-content in 50 samples at week 8 was performed, presented in table 8. Mixing the juice did not change the relationship between measurements of dissolved oxygen measured in one of two ways. Measuring with and without mixing the juice displayed strong correlation (P = 0,000).

3.4 VITAMIN C CONTENT

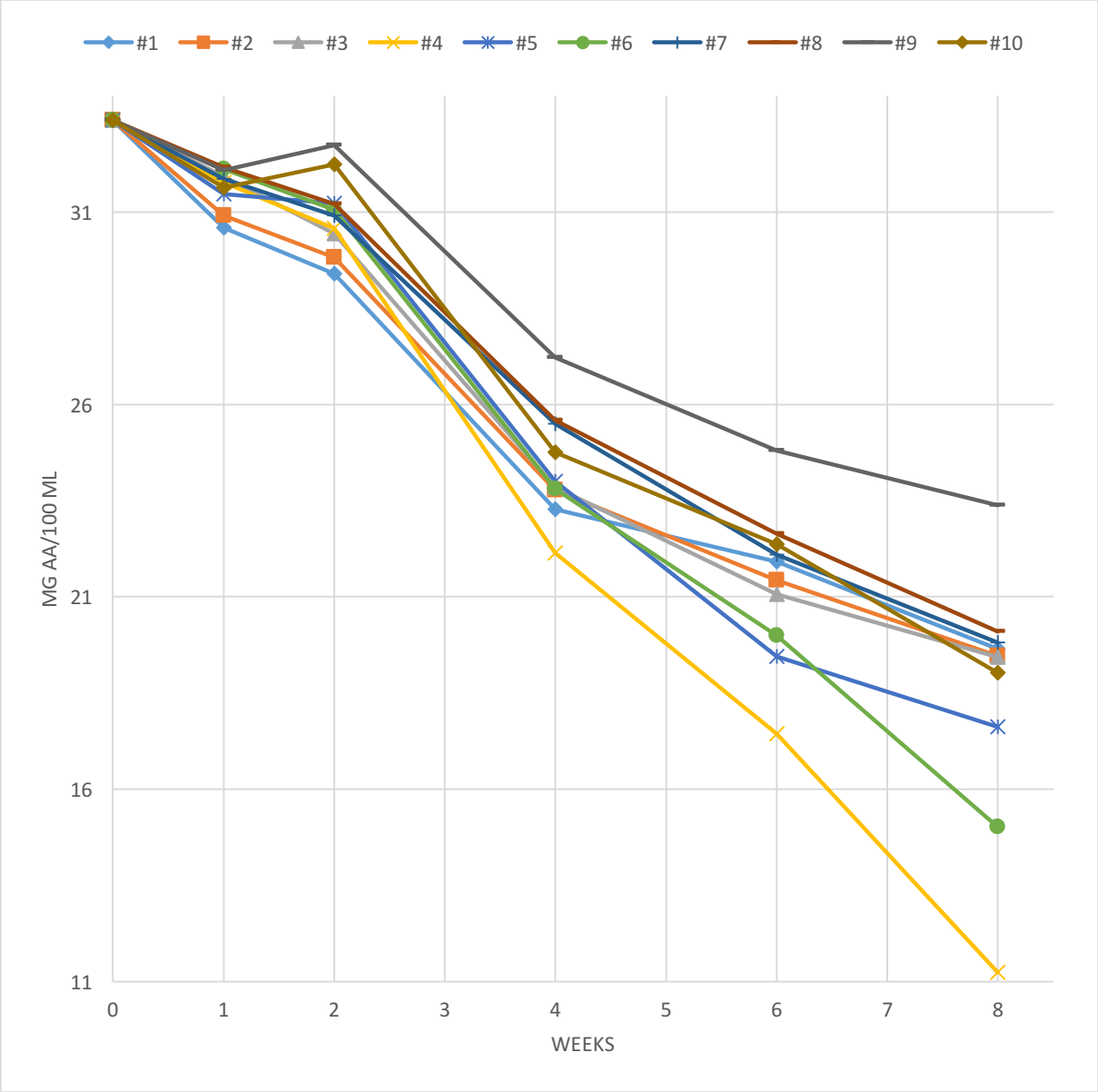

Figure 11: Vitamin C-content development in orange juice, stored in gable top cartons with and without barrier imperfections over the duration of 8 weeks. Each parallel represents an average of 5 cartons tested.

Development in vitamin C content measured in orange juice is presented in Figure 11. All samples displayed a reduction in vitamin C content, with the lowest content found in sample 4, 5 and 6 at 8 weeks of storage. Samples 4, 5 and 6 were cartons with barrier imperfections along the 1. or 5. panel. Table 9 contains numeric values for dissolved oxygen content measured over 8 weeks, with standard deviation. Letters in Table 7 represents statistical significant difference (Tukey test, CI = 95%), where samples presented with the same letter are not different.

Table 9: Vitamin C content measured in orange juice, stored in gable top cartons with and without barrier imperfections, with standard deviation. Samples tested over 8 weeks, each value represents an average of 5 cartons tested. At Day 0, all samples contained 33,39±0,12 mg vitamin C per 100 ml juice. Letters indicate differences, samples with the same letters where not statistically different in a Tukey test. CI: 95%.

Sample	Week 1	Week 2	Week 4	Week 6	Week 8
	30,59±0,12	29,40±0,45	23,27±1,16	21,92±0,26	19,64±0,27
#1	C	E	DE	BC	B
	30,91±0,21	29,82±0,13	23,77±0,37	21,43±0,71	19,47±0,94
#2	BC	DE	CD	CD	B
	31,92±0,30	30,44±0,33	23,85±0,27	21,06±0,27	19,43±0,40
#3	A	CD	CD	D	B
	31,77±0,61	30,57±0,57	22,14±0,97	17,44±0,57	11,24±0,72
#4	A	BC	E	F	E
	31,47±0,34	31,23±0,40	24,00±0,47	19,45±0,17	17,62±0,33
#5	AB	B	CD	E	C
	32,11±0,45	31,05±0,39	23,80±0,36	20,00±0,61	15,02±0,88
#6	A	BC	CD	E	D
	31,86±0,19	30,90±0,41	25,49±0,36	22,09±0,65	19,81±0,59
#7	A	BC	B	BC	B
	32,17±0,46	31,21±0,26	25,59±0,67	22,64±0,42	20,10±0,90
#8	A	B	B	B	B
	32,09±0,63	32,73±0,24	27,23±0,26	24,81±0,25	23,37±0,36
#9	A	A	A	A	A
	31,63±0,25	32,24±0,09	24,76±0,36	22,37±0,30	19,03±1,64
#10	AB	A	BC	BC	BC

A sample evaluated at week 4 on one carton showed a difference in the vitamin C amount in the top and bottom of the carton to be 23,6%. This indicated that the juice sample in the carton had to be mixed before the sample was taken. Vitamin C content measured decreased from 29,5 mg/100 ml in the bottom to 22,55 mg/100 ml in the top. The measured value after mixing the juice was 24,9 mg/100 ml.

3.5 AOIR

Figure 12: Oxygen transfer rate (OTR, ml O_2/pack/day) measured in EVOH and aluminum gas barrier gable tops with and without gas barrier imperfections. Average with standard deviation.

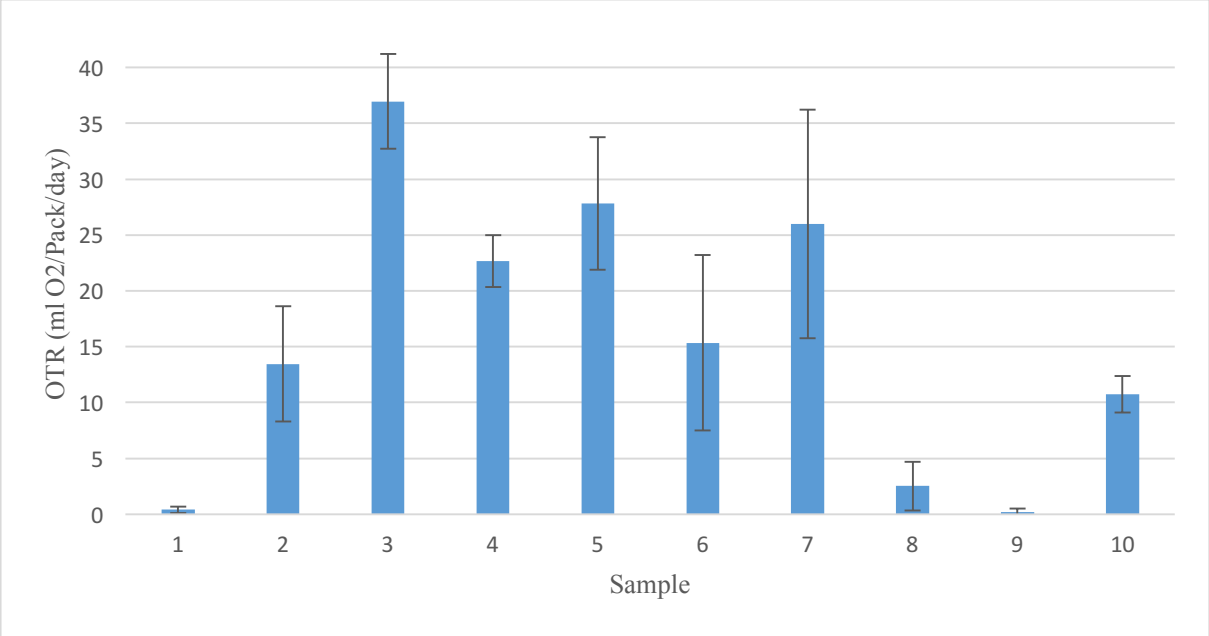

Table 10: Tukey pairwise comparison test of OTR (ml O_2/pack/day) measured in EVOH and aluminum gas barrier gable tops with and without barrier imperfections. Samples grouped with the same letter was not different at 95% confidence.

Sample	Mean	Grouping
1	0,40 ± 0,28	E
2	13,46 ± 5,16	D
3	36,97 ± 4,22	A
4	22,64 ± 2,31	BC
5	27,84 ± 5,94	AB
6	15,38 ± 7,87	CD
7	25,98 ± 10,24	B
8	2,52 ± 2,17	E
9	0,19 ± 0,30	E
10	10,75 ± 1,63	CDE

A correlation test of AOIR and vitamin C resulted in no significant correlation (P = 0,264).

There was observed a significant difference in OTR measured with the AOIR-method in several samples. Samples 1, 8, 9 and 10 were not significantly different.

3.5.1 Volume determination of gable tops

The PermMate system was used to measure the gable top carton volume. An average of 10 gable tops had a volume of 73,84 ml, with a relative standard deviation of 5,4%. This value was then used in all gable tops for calculating AOIR.

3.6 Dye Test area

Table 11: Measured dye fluid penetration area on board in EVOH gable top cartons (mm^2)

Sample	Dye area (mm^2)	SD
2	628,20	211,39
3	1851,60	1590,58
4	69,77	41,71
5	74,33	30,92
6	43,03	18,13
7	1,12	0,4
8	0,76	0,37

A correlation test of AOIR and dye spot area(mm^2) resulted in no significant correlation (P = 0,169). Measured area of dye fluid penetration (mm^2) on board in gable tops 2, 3, 4, 5, 6, 7 and 8 are presented in table 11. Sample 1 and 9 did not display any dye spots (A = 0,0 mm^2).

3.6.1 Correlation of vitamin C content and area of dye penetration

A correlation test between dye penetration area in gable top and vitamin C in 15 cartons was performed. Samples used were 2, 3, 4, 5 and 6 with 3 gable tops from each sample.

The test returned a significant result with P = 0,036 and a weak correlation of 0,484. Meaning that a larger dye penetration area correlated with a higher content of vitamin C.

3.7 Microscopy of manual made barrier imperfections

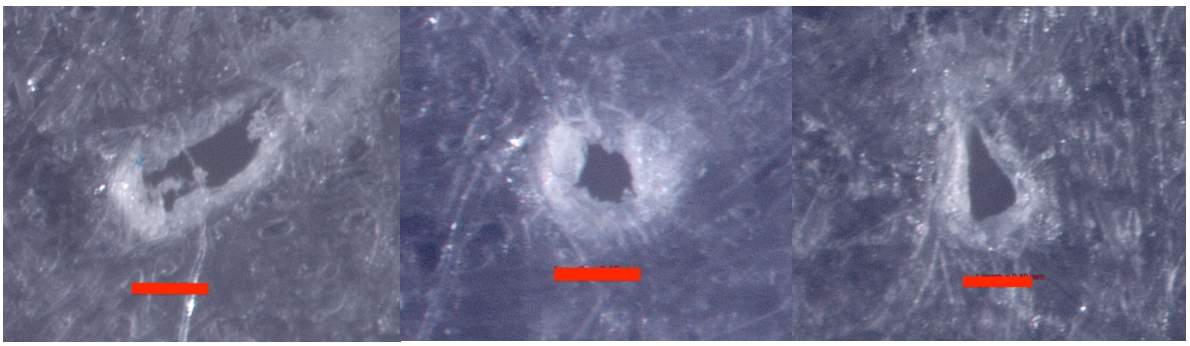

Figure 13: 100 µm manual made barrier imperfections in EVOH barrier layer in 3 EVOH gas barrier cartons after removal of board. Line: 100 µm.

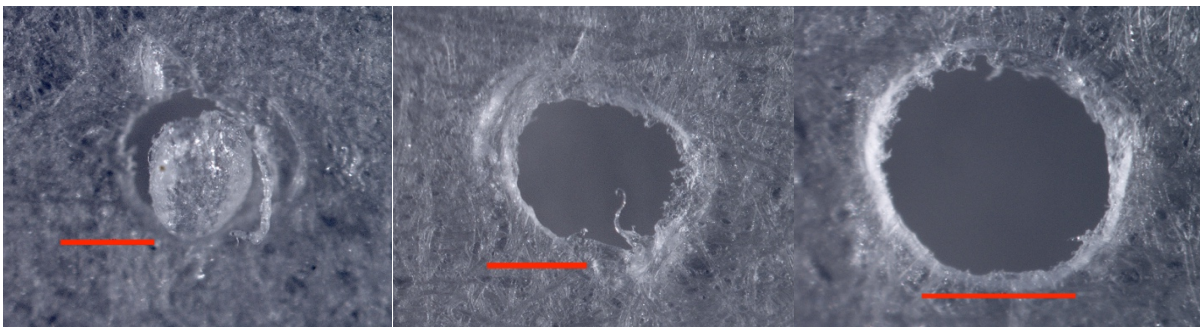

Figure 14: 500 µm manual made barrier imperfections in EVOH barrier layer in 3 EVOH gas barrier cartons after removal of board. Line: 500 µm.

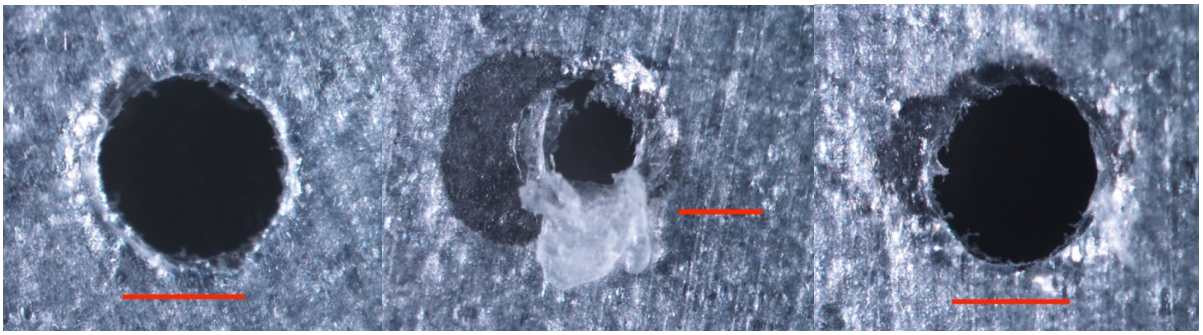

Figure 15: 500 µm manual made barrier imperfections in aluminum barrier layer in 3 aluminum gas barrier cartons after removal of board. Line: 500 µm.

Images of manual made barrier imperfections in EVOH barrier layer in 3 EVOH gas barrier cartons (sample 2) after removal of board inspected and photographed in microscope are displayed in Figure 13, where the red line represents 100 µm. Images of manual made barrier imperfections in EVOH barrier layer in 3 EVOH gas barrier cartons (sample 3) after removal of board inspected and photographed in microscope are displayed in Figure 14, where the red line represents 500 µm. Images of manual made barrier imperfections in aluminum barrier layer in 3 aluminum gas barrier cartons (sample 10) after removal of board inspected and photographed in microscope are displayed in Figure 15, where the red line represents 500 µm.

3.8 Sensory analysis

Table 12: Results of two triangle tests with 10 panelists. **1.** Reference aluminum gas barrier compared to EVOH gas barrier with imperfections on 1. panel. **2.** Reference EVOH gas barrier compared to EVOH gas barrier with imperfections on 1. panel. CI: 95%

Test	Answers taken	Answers right	Significance
1. Reference aluminum gas barrier / EVOH gas barrier with imperfections on 1. panel.	10	7	0,0197
2. Reference EVOH gas barrier / EVOH gas barrier with imperfections on 1. panel.	10	2	0,8960

Table 12 are results from two triangle tests with 10 panelists. Test 1. is a reference aluminum gas barrier (Sample 9) compared to EVOH gas barrier with imperfections on 1. panel (Sample 4). Test 2 is a reference EVOH gas barrier (Sample 9) compared to EVOH gas barrier with imperfections on 1. panel (Sample 4). CI: 95%

3.9 BALANCED ANOVA

In EVOH-gable top cartons with barrier imperfections the type of barrier imperfection explained 3,51% of the effect and storage time explained 91,18% of the effect on the change in vitamin C. Interaction effect (BI*WN) was low, and explained only 4,6% of the effect. A higher effect was found in type of barrier for dissolved oxygen with 12,88% of the effect caused by type of barrier imperfection. The barrier effect on headspace oxygen concentration was 55,18%, with little effect of week number (storage time).

Table 13: Overview of balanced ANOVA results for the responses and main effects BI (Barrier Imperfection), WN (Storage time) and interaction BI*WN in EVOH carton samples with barrier imperfections (8*5). The numbers are explained variance (in %). All values were significant for a 5% significance level.

	Vitamin C	Dissolved O_2	Headspace O_2
Type of barrier imperfection (BI)	3,51 %	12,88%	55,18%
Week number (WN)	91,18%	51,56%	7,31%
BI*WN	4,60%	13,11%	22,48%
Residuals	0,71%	22,43%	14,96%
R^2 adj.	99,12%	72,09%	81,34%

An effect from type of barrier was significant on the vitamin C and dissolved oxygen content in orange juice as well as headspace oxygen concentration. Although most of the effect on vitamin C content, dissolved oxygen content and headspace oxygen concentration was accounted to the storage time, with 85,43%, 75,93% and 86,90%, respectively.
12,56% of the effect on vitamin C deterioration in orange juice could be accounted to the variation in permeability in EVOH and aluminum barrier gable top cartons.

Table 14: Overview of balanced ANOVA results for the responses and main effects BI (Barrier Imperfection), WN (Storage time) and interaction BI*WN in EVOH and aluminum carton samples with **no barrier imperfections** (2*5). The numbers are explained variance (in %). All values were significant for a 5% significance level.

	Vitamin C	Dissolved O_2	Headspace O_2
Type of barrier imperfection (BI)	12,56%	1,28%	8,72%
Week number (WN)	85,43%	75,93%	86,90%
BI*WN	1,00%	12,58%	1,85%
Residuals	0,99%	10,15%	2,47%
R^2 adj.	98,78%	87,54%	96,96%

In EVOH and aluminum gas barrier cartons with 500 µm manual made barrier imperfections the effect of the different samples was low for vitamin C and dissolved oxygen content. The samples had an higher effect on headspace oxygen, with 9,06%.

Table 15: Overview of balanced ANOVA results for the responses and main effects BI (Barrier Imperfection), WN (Storage time) and interaction BI*WN in EVOH and aluminum carton samples with 500 µm manual made barrier imperfections. (2*5). The numbers are explained variance (in %). All values were significant for a 5% significance level.

	Vitamin C	Dissolved O_2	Headspace O_2
Type of barrier imperfection (BI)	0,42%	2,81%	9,06%
Week number (WN)	97,80%	58,00%	16,35%
BI*WN	0,71%	23,06%	10,22%
Residuals	1,05%	16,00%	64,31%
R^2 adj.	98,70%	80,29%	21,19%

3.10 Correlation of vitamin C-content, dissolved Oxygen content and headspace Oxygen concentration

Significant strong correlation between vitamin C content and dissolved oxygen content in the juice was found in sample 1, 5, 6, 8, 9 and 10. Significant moderate correlation was found in sample 2, 4 and 7. Sample 3 displayed weak correlation of vitamin C content and dissolved oxygen content.

Significant strong correlation between vitamin C content and headspace oxygen concentration was found in sample 1, 8 and 9. Other samples displayed low correlation in headspace oxygen concentration and vitamin C content, where only sample 3 and 10 were significant.

Significant strong correlation between headspace oxygen concentration and dissolved oxygen content were found in sample 1, 8 and 9.

Table 16: Correlation analysis of vitamin C content, dissolved Oxygen content and headspace oxygen concentration 10 sample parallels during storage for 8 weeks. Gable top cartons with EVOH and aluminum gas barrier samples. Significant strong correlation indicated in dark green and significant medium correlation indicated in light green.

Sample 1	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,814 P-value: 0,000	
Headspace O_2	Correlation: 0,956 P-value: 0,000	Correlation: 0,824 P-value: 0,000

Sample 2	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,620 P-value: 0,001	
Headspace O_2	Correlation: 0,156 P-value: 0,455	Correlation: 0,317 P-value: 0,123
Sample 3	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,533 P-value: 0,006	
Headspace O_2	Correlation: -0,398 P-value: 0,049	Correlation: 0,010 P-value: 0,961
Sample 4	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,615 P-value: 0,001	
Headspace O_2	Correlation: -0,268 P-value: 0,195	Correlation: 0,019 P-value: 0,929
Sample 5	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,905 P-value: 0,000	
Headspace O_2	Correlation: -0,173 P-value: 0,409	Correlation: 0,120 P-value: 0,567
Sample 6	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,895 P-value: 0,000	
Headspace O_2	Correlation: 0,263 P-value: 0,204	Correlation: 0,296 P-value: 0,151
Sample 7	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,677 P-value: 0,000	
Headspace O_2	Correlation: 0,064 P-value: 0,763	Correlation: 0,148 P-value: 0,481
Sample 8	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,791 P-value: 0,000	
Headspace O_2	Correlation: 0,831 P-value: 0,000	Correlation: 0,689 P-value: 0,000
Sample 9	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,866 P-value: 0,000	
Headspace O_2	Correlation: 0,952 P-value: 0,000	Correlation: 0,875 P-value: 0,000
Sample 10	Vitamin C	Dissolved O_2
Dissolved O_2	Correlation: 0,902 P-value: 0,000	
Headspace O_2	Correlation: -0,435 P-value: 0,030	Correlation: -0,453 P-value: 0,023

4. DISCUSSION

4.1 VITAMIN C CONTENT IN ORANGE JUICE AT DAY 0

An issue in this investigation was the fact that the juice concentrate used contained 35 mg vitamin C per 100 ml of juice diluted to 11,3 brix before pasteurization. This in contrast to the usual 40-42 mg/100 ml of vitamin C in orange juice delivered at Elopak when performing shelf life tests (Andre Dybvik, Elopak, 26.9.16). Although, vitamin C content in the juice delivered was within the product specification for the provider of the juice concentrate, which was at 35 mg/100 ml. This means that the level of Vitamin C in the product at the different times of testing was possibly not representative of what is usually the vitamin C content in a real-world product. On the contrary one could argue that the testing should be performed at just the lower level of the product specification to observe the absolute worst-case scenario that the producer could experience. Then the vitamin C amounts found in this thesis is representative of the worst case in the industry.

After 6 weeks sample 4 and 5 contains vitamin C below the lower content limit set by EU, two weeks before the juice expired, but at a storage temperature that is higher than usual in a product bought by a consumer. After 8 weeks when the juice would have expired sample 4, 5, 6, 10, 3 and 2 where all under the lower content limit set by EU. Both reference samples with aluminum and EVOH barrier contained over 19,5 mg/100 ml juice, although the EVOH carton contained the least vitamin C.

4.2 VITAMIN C DETERIORATION

At week 1 and 2 the juice was not mixed before sample outtake, where the sample was taken from the bottom of the carton. This resulted in higher observed values for vitamin C than what was the average for the juice content of the carton. As demonstrated less dissolved oxygen was present in the bottom of the carton, thus less oxidation occurred and the measured vitamin C was higher than it would have been if the juice was mixed before the measurement. The difference in Vitamin C in the bottom and the top of the carton showed that mixing the juice before the measurement was essential for the analysis to provide a result representing all the juice in the carton.

Cartons in sample 1 and 9, reference with EVOH- and aluminum-barrier, saw a decrease of vitamin C of 34,4% and 25,7% after 6 weeks, respectively. The difference increased 22,6% to 30,0% and 41,2% after 8 weeks, respectively. The difference in vitamin C

in the reference EVOH- and aluminum-barrier cartons suggested a difference in permeability in the different barriers, as oxygen in the headspace and dissolved in the orange juice was the same at day 0. EVOH with no barrier imperfections contained significantly more vitamin C at 8 weeks than sample 4, 5 and 6 wile sample 9 contained significantly more vitamin C. The EVOH-reference then contained an amount of vitamin C, not significantly different from sample 2, 3, 7, 8 and 10. This indicated that the barrier imperfections in samples 2, 3, 7, 8 and 10 did not have an impact on the vitamin C-content compared to a carton with no imperfections.

At 8 weeks, sample 4, 5, and 6 contained significant less vitamin C than the other EVOH-barrier cartons. These cartons had barrier imperfections that were produced by heat on the 1. or 5. panel in a converter, indicating a more severe contribution to OTR from the barrier imperfections produced by heating the gas barrier, compared to barrier imperfections made manually. The results of sample 3 indicated no difference from the reference at week 4 and 8, although a marginal difference at week 6. This means that even a 500 μm perforation in the EVOH gas barrier in a carton does not deteriorate vitamin C-content.

The sensory analysis at week 8 indicated that it would be highly difficult to taste a difference of 8,4 mg vitamin C/100 ml, tested on EVOH-barrier cartons with the most and least vitamin C at week 8. Although, 8 out of 10 panelists tasted a difference in the triangle test comparing sample 9 and sample 4. This implied that the difference in two samples most consumers could taste was between 8,40 and 12,13 mg vitamin C per 100 ml juice.

Correlation of area on board after dye testing and vitamin C content of juice in the sample displayed a positive, although weak, correlation ($P = 0,036$). This indicated that when the dye spot increased in area the vitamin C content in the sample could be expected to increase. As a larger dye spot area was associated with a more severe barrier imperfection, the results were inverse of what was expected. A possible explanation could be the different behavior of dye fluid in a manual made and naturally formed barrier imperfection. The correlation test pooled natural and manual made perforations. Manual made perforations created a channel directly to the board through all polymer layers inside the carton whereas natural formed perforations were created by heat, and were irregular. The irregularity could be caused by different melting temperatures in polymers and marginally different thickness in different areas. This could form channels through the polymers varying in size, shape and direction, decreasing dye penetration.

Temperature recorded in 2 products had an average difference of 0,73°C. This was not enough for significantly affecting the vitamin C content in the samples per previous tests at

Elopak (Elin Margrethe Johansen, Elopak, 2.12.16). The average temperature recorded in the cooling chamber of 7,78±0,64°C was satisfactory as it was close to the target temperature.

4.3 Headspace Oxygen concentration

As vitamin C in the juice is oxidized by dissolved oxygen, oxygen in headspace would migrate to the orange juice to achieve equilibrium, reducing the concentration of Oxygen in the headspace atmosphere, it there were no barrier imperfections. In a carton with perforations in the gas barrier, oxygen would migrate from the ambient atmosphere and little to no reduction in oxygen concentration in the gable top would occur.

The oxygen concentration in the gable tops decreased as expected in the references, sample 1 and 9. The difference in sample 1 and 9 indicated that an aluminum barrier possesses better barrier capabilities than a EVOH-barrier.

oxygen concentration in the headspace of gable tops in all the cartons with perforations remained over 17,5%, except from sample 8. In sample 8 the standard deviation indicated that the effect was systematic. Under-heating of a gable top during production can be one of the less serious problems regarding carton integrity. In samples 4, 5, 6, 7 and 8 it could be expected to be fluctuations because of the irregular nature of natural forming barrier imperfections. The barrier imperfections could be different from carton to carton, due to plastics melting irregularly under heat treatment or due to product filling perforations. Headspace oxygen concentration in perforated cartons indicated a high enough contribution to the total permeability of the carton from the perforations, that the orange juice had a practically unlimited supply of O_2.

Samples 1, 9, 4 and 10 where expected to be predictable and systematic as the 500 µm perforations never where observed to be plugged by elements in the juice. Although as microscopy of perforations revealed, there could be differences in the structure of perforations from carton to carton, that could have had an influence on permeability.

Sample 4 and 10 had the highest average headspace oxygen concentration week 4 and 6. The low value and high standard deviation of sample 10 at week 2 was explained with an outlier in the five parallels without proper perforation.

For headspace oxygen concentration to decrease during storage the permeation of the carton with possible barrier imperfections must be lower than oxygen consumed by oxidation of vitamin C in the orange juice. At a higher temperature, where Vitamin C deterioration

would be greater (Klimczak et al. 2006) the headspace oxygen concentration could possibly be reduced compared to this test.

4.4 Dissolved Oxygen content

Per Elopak method the concentration of dissolved oxygen was measured with the sensor static in the juice and with no stirring or mixing prior or during the measurement. In a paper investigated where dissolved oxygen was measured in juice, the juice was mixed carefully while the sensor was in the juice either during or before the measurement of dissolved oxygen (Solomon, Svanberg, 1995). A test with 10 samples was performed week 6 where dissolved oxygen was measured with static juice and again after mixing carefully in the carton with a glass rod. The difference in results indicated that further testing was necessary. In the 50 samples tested week 8 the average decrease in dissolved oxygen (48%) from all the measurements indicated a higher content of dissolved oxygen in the upper part of the juice in the carton and less in the bottom of the carton. Dissolved oxygen in static juice appears to not even out towards the bottom of the fluid as oxygen dissolves in the top of the fluid. This then created a gradient from the top to the bottom of the juice in the carton with less dissolved oxygen further down, perhaps caused by the viscosity of the orange juice. The dissolved oxygen gradient can may be assumed to be opposite of vitamin C content, where the highest content of vitamin C can be found in the bottom of the fluid.

The measurements in this project was performed with no mixing of the juice and was therefore subject to random mixing of the juice during transport of the juice to the lab from cold storage and handling of the carton before the measurement. The values were probably higher than the average for the entire juice content and can then only be interpreted as indications of the dissolved oxygen levels. This also explains the cartons where it was observed an increase in the dissolved oxygen level from week 4 to week 6. This effect appeared as strong in cartons with low and high permeation in the package. A package with less O_2 in the gable top, by flushing in production, could possibly see a lower gradient of dissolved O_2 from the top to the bottom of the fluid level in an intact carton.

The strong correlation of dissolved oxygen content before and after mixing of 0,827 (P = 0,000) indicated that measurements of dissolved oxygen before mixing could be used to explain possible differences in samples, but not the content of dissolved oxygen in the sample.

The strong correlation of dissolved oxygen and vitamin C deterioration in sample 1, 2, 4, 5, 6, 7, 8, 9 and 10 was in accordance to what was expected (P = 0.000 (2 & 4: P = 0,001)). As vitamin C oxidized, less oxygen would be present in the orange juice. Lack of strong

correlation in sample 3 could be due to dissolved oxygen content measurement not being accurate, as the measured content increased from week 4 to week 6. Sample 1 and 9, where headspace oxygen concentration decreased to under 9% had a strong correlation with vitamin C deterioration (P = 0,000). This was expected as oxygen in headspace migrated to the orange juice due to reduced content in the juice, to maintain equilibrium. And per the dye test with no faults, little to no oxygen could permeate the packages after filling. Cartons with perforations did not show a correlation between vitamin C content and headspace oxygen, due to oxygen permeating barrier imperfections as oxygen in the headspace dissolved in the juice.

4.5 AOIR

There are multiple factors to discuss concerning development of an AOIR-procedure for a gable top carton;

1. Data recorded with the AOIR method in this thesis.
Results of OTR measurements showed a high degree of irregularity. This was to an extent expected, as the perforations were irregular, as determined visually with dye testing.
The high relative standard deviation (sample 1: 70% and sample 9: 157%) in cartons with no barrier imperfections was not expected as the method has been confirmed to provide values with low standard deviation in plastic films and thermoformed plastic trays (Larsen et al. 2000, Larsen et al. 2002). The method was able to differentiate the average permeability of the cartons, although the high standard deviation indicated a high sample selection should be chosen when using the method. The lack of significant correlation between measured OTR and vitamin C content indicated that there could have been permeability differences in the cartons below fluid level, that the method of measuring gable tops only would not register. This was due to the assumption that perforations below fluid level did not high degree influence the permeability of the package.

2. Storage temperature, with and without barrier imperfections.
When testing a carton gable top with barrier imperfections with the AOIR method the sample might not need to be stored at cooling temperature during conditioning as it is not likely to have an impact on the OTR, based on the findings of Hanne Larsen (2004). This eliminates one of the argued advantages of the AOIR-method compared to OxTran. In a carton with no barrier imperfections, gas permeation through the gas barrier is the only route. In such a case

samples, should be stored in a temperature relevant to the test, as the temperature will have an impact on the gas transmission rate (Larsen 2004).

3. Storage time having an influx on the measurements.

This method has been proven to work for packages of very low permeability (Larsen et al. 2000), but has not been validated properly on packages with higher OTR or gable top cartons. With this taken into consideration the AOIR of a gable top was tested at different intervals and a pattern of being provided with different results at different measurement intervals appeared. A measurement from 2-4% oxygen in headspace resulted in a higher value than that from for example 5-12% oxygen in headspace. This can perhaps be explained in part by the curve in figure 14, although the formula is designed to take the nonlinearity of OTR depending on concentration difference into account. For this experiment the gable tops had perforations in the gas barrier and the filling of the cartons happened without flushing. This means that the start oxygen-value in the headspace was ambient oxygen level. With the perforations and the consequential high OTR this oxygen level never decreases much, except from in the reference cartons. This information makes it possible to argue that the AOIR-measurements most relevant to real-life should be performed at the highest interval possible or at least above 12-15%.

The volume increase effect, where O_2 migrates through a barrier at a ratio of 4:1 compared to N_2 could decrease the relative concentration of oxygen in a package over time. This argues that measurements should be performed as quickly as possible.

4. OTR in a gable top carton with and without orange juice.

The gas permeability in a "real life" situation with an oxidizing product in a carton can perhaps be different from the measurements in a lab with an empty gable top. This because the oxygen will oxidize Vitamin C and therefore the concentration in the juice will decrease. Decreased dissolved oxygen in the orange juice will mean that the juice must absorb oxygen from the headspace to achieve equilibrium. Lower oxygen concentration in the headspace will move the permeability to the left on the curve in Figure 16. and therefore, possibly increase gas transmission rate. It might be possible to take this into account in the calculation of OTR if the amount of oxygen consumed by the product was known.

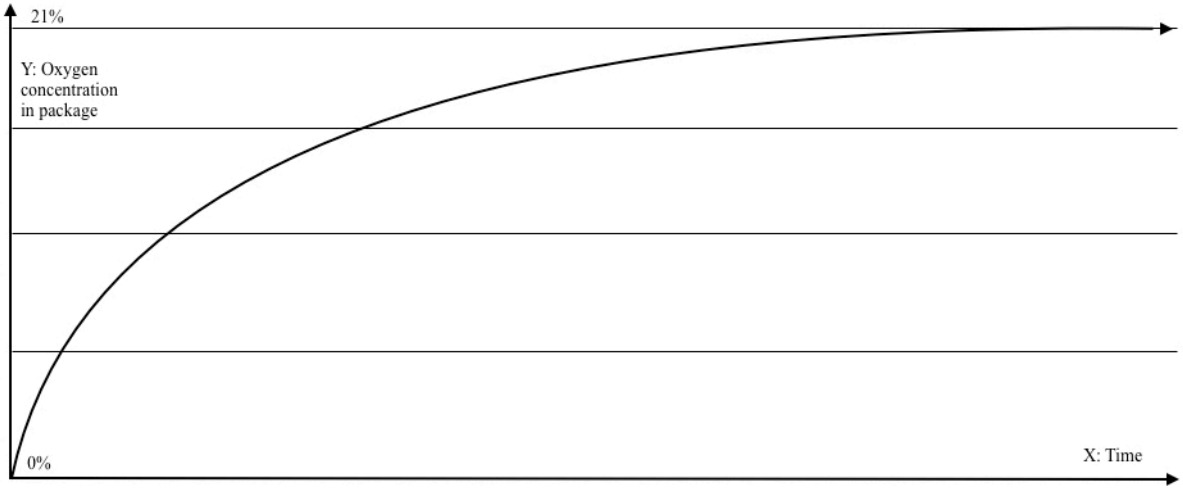

Figure 16: Illustration of oxygen transfer rate from ambient atmosphere to atmosphere in a package, depending on oxygen concentration in the package when the ambient atmosphere contains 21% oxygen. As concentration in package headspace increase the differential pressure decreases and oxygen transfer rate decreases. Slope of the curve indicates TR.

5. Practicality with regards to irregularity of barrier imperfections.

Natural formed gas barrier imperfections in a gable top carton can be highly irregular. Methods available today can find if a barrier imperfection is present in a carton and describe the structure with observations in a microscope but not determine the accurate OTR contribution belonging to a perforation. Developing a reliable AOIR-method for use on gable top cartons can be a valuable tool to determine the severity of a barrier imperfection.

6. No correlation of dye and OTR.

The lack of correlation between dye spot area and OTR measured with AOIR, could be seen in relation to the lack of correlation between dye spot area and vitamin C deterioration. Dye fluid and gas could behave differently, and migrate through a perforation at different rates, depending on the characteristics of the perforation.

7. Fastening gable tops to a surface with epoxy glue.

A method for rendering the bottom of the gable top separated from the carton air tight in the bottom had to be developed. Fastening the gable top to an air tight metal foil was efficient and allowed for multiple samples to be tested in the same run. The foil was advantageous compared to a steel plate, where epoxy glue would adhere much stronger, to the point where the gable top could not be removed intact for dye-testing.

As the PermMate manual suggests, the septum could be placed on the bottom of a package where the opening was covered with a non-permeable material. This was unpractical for two

reasons; 1. The light adhesion of epoxy to the metal foil suggested it could loosen during manipulation for performing measurements. 2. Stacking and moving gable tops upside-down could present with further challenges.

8. The formula

$$TR = -\frac{V}{t_f - t_i} \times ln\left(\frac{C_{air} - C_f}{C_{air} - C_i}\right) \quad (1)$$

The formula (1) the AOIR-method is based on could possibly be used for calculating OTR of gable top cartons without the use of a PermMate II-system, but this would require further research, as there was not sufficient time to compare the two methods.

4.5.1 Removal of board on gable tops

Several options for attachment of the septum to the gable top was explored as the AOIR-method was not previously used on a gable top carton. Placement on the outer PE-layer was not an option as gas will travel through the PE and board, under the septum and through the perforation in the EVOH-gas barrier. Illustrated in figure 15. Determined in a preliminary test, measured OTR decreased to a hundredth with removal of the board and PE. The most secure and reliable attachment of the septum was determined to be directly in the gas barrier under the board after the board was completely removed.

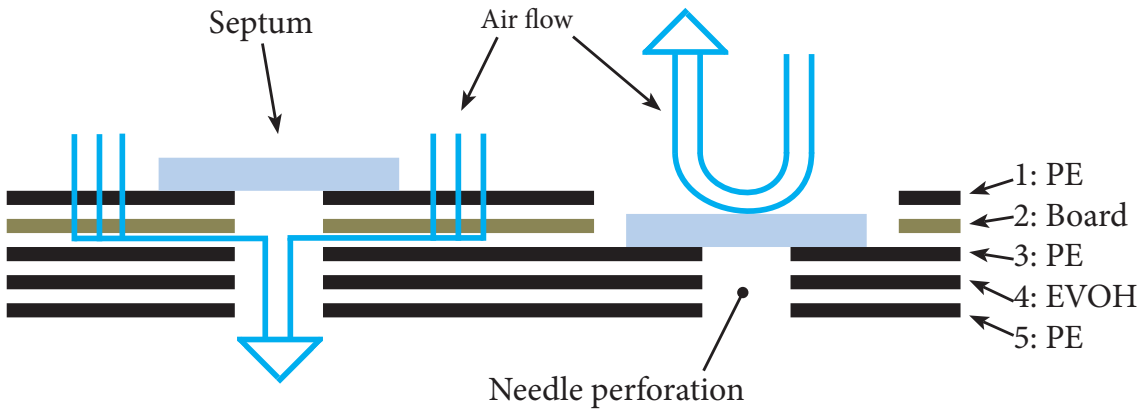

Figure 15: Illustration of gas route in carton layers under septum without removal of outer PE and board layer to the left. To the right septum placement after board and PE removal.

Attachment on the closure was used as an alternative method so the removal of board would not interfere with the dye test or AOIR at a later time. The glue on the septum did not affix properly to the lid and epoxy had to be used to secure the attachment. Although with the introduction of several insertions of the needle the brittle epoxy could break of the lid and cause leakage. A leakage would be visible on the lid and the problem could be solved by adding more glue.

The removal of the board and PE could have influenced the OTR measured with AOIR, depending on the characteristics of the perforations. If a barrier imperfection was in the vicinity of the area where the board and PE was removed, gas could possibly gain another route with less resistance to perforations in the gas barrier. This could be an argument for always placing the septum on the gable top lid when working with cartons, to stay as close to the original package properties as possible.

4.6 MICROSCOPY OF GAS BARRIER IMPERFECTIONS

Images of 100 μm barrier imperfections in EVOH-cartons exhibited irregularities in the barrier perforation size and shapes. This could influence the permeability of each perforation. The oblong barrier imperfections may be caused by the needle tip being slightly bent during operation causing the irregular perforations. Previous needles where observed in microscope to be slightly bent after use. Per Allan-Wojtas et al., (2008) material residue in barrier imperfections may cause differences in OTR for a plain plastic film. Irregularities in hole structure and material residue partially blocking the perforations was the case for some of the barrier imperfections in this study, as seen in Figures 13, 14 and 15. The difference in the structure and size of the perforations might have had an impact on the OTR contribution of the barrier imperfections on the total OTR. Although in this project the barrier imperfections was not directly exposed to air and the findings of Allan-Wojtas et al. might not be applicable.

As Allan-Wojtas et al. found in 2008, Barrier imperfections larger than 55 μm in diameter may not have a predictable OTR as smaller barrier imperfections had. A barrier imperfection with an area of 6500 μm^2 had previously been measured to result in an OTR of 115-135 in a film (Wojtas et al., 2008). In this test the barrier imperfections was a part of the structure in a gable top carton and not in direct contact with the atmosphere. A layer of board as well as an outer layer of PE was still intact on the outside of the carton and the OTR might be lower because of this.

4.7 JUICE FILLING BARRIER IMPERFECTIONS

An effect that had to be taken into consideration during the experimental design phase was the fact that elements in the orange juice could potentially "plug" barrier imperfections in the inner layers in the carton. Experimentation with this effect and different sized barrier imperfections found that 100 µm barrier imperfections could be sealed just by contact with the juice. Meanwhile 500 µm barrier imperfections where never filled in testing in this case. Therefore, it was deduced that the barrier imperfection size that was the smallest possible, while at the same time not plugged by juice were in the interval 100-500 µm. A tool to produce barrier imperfections in the carton material reliably and consistently between those extremes could not be produced in the timeframe available. Further experiments to find the cut-off size where a barrier imperfection always stays open in contact with material in juice would be recommended in the future. What materials in the juice that are filling barrier imperfections in the carton material are assumed to be sugars in the orange juice. Related to this issue is also the assumption that barrier imperfections that are below the fluid level in the carton will have little to no effect on the total permeability of the package and therefore no effect on the quality of the product. This effect could be studied by filling a carton with barrier imperfections at different heights along the carton with a de-aerated fluid and comparing the OTR with that of an identical carton with no fluid inside. This protection provided by the product in the carton is also important for the EVOH-carton experimented with in this project, as the bottom Japan-type seal is not always gas-tight.

5. CONCLUSION

A certain amount of barrier imperfections does not influence the vitamin C content in juice stored in a EVOH gas barrier gable top carton, even after 8 weeks of storage. Suggesting a EVOH gas barrier carton does not need to be perfect to retain vitamin C content. This implies that a EVOH gas barrier carton could present with a positive result in a dye test in a production facility and still retain the desired content of vitamin C in orange juice up to the expiration date. A spot from dye test can still result in a perfect product if the perforation stays open. Suggesting the area of a dye spot cannot be used as an indicator of the severity of a barrier imperfection but only as a quantitative analysis in the future.

Measuring OTR with AOIR could prove promising as a method for determining permeability of a gable top carton as tests with the PermMate did provide different results in

multiple samples. The selection of barrier imperfections in the project were not ideal for verification of the method as there was a high degree of variation in the permeations tested. Test setup and time constraints, limited the possibility of further testing of the AOIR-method for adjustments to the procedure or verification.
As barrier perforations that form in cartons have a high degree of variation and are highly unpredictable, it is difficult to emulate real barrier imperfections.

The registered content of dissolved oxygen in the orange juice in this project, was different than the actual dissolved oxygen content for an average of the juice content. This indicated that the dissolved oxygen content could not be considered when assessing the absolute quality of the juice, only the quality of the samples relative to each other. This was confirmed by the strong correlation between measurement of dissolved oxygen before and after mixing the orange juice.

In preparation of a project where the objective is to study gable top cartons, the primary concern should be to obtain the maximum amount of knowledge possible about the functionality and characteristics of the carton before planning of the test scheme. In this thesis, new information and realizations about the carton and orange juice characteristics changed the premises of some tests after testing had commenced. A different quality in the data collected could be achieved with further knowledge of the carton and orange juice characteristics before commencing the practical work. Still a great amount of data was collected, which could prove useful for further understanding of the mechanics of a gable top carton.

6. SUGGESTIONS FOR FURTHER WORK

If cartons with a linear increase in permeability caused by perforations were tested for vitamin C degradation in orange juice over time, this could determine a cut off level of OTR contribution from perforations in EVOH gas barrier for vitamin C deterioration to increase.

Test for correlation between vitamin C content and the permeability of an entire EVOH gas barrier carton, measured with the AOIR-method. This could confirm or deny the assumption that barrier imperfections below fluid level does not affect the deterioration of vitamin C in orange juice during storage.

Test the difference in results when OTR is calculated with the use of the formula (1) the AOIR-method is based upon and when calculated with the PermMate system.

$$TR = -\frac{V}{t_f - t_i} \times ln\left(\frac{C_{air} - C_f}{C_{air} - C_i}\right) \quad (1)$$

Dissolved oxygen development at different depths in orange juice in cartons with and without barrier imperfections could be investigated in relation to the practice of flushing gable top headspace with H_2 during filling.

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APPENDIX

ATTACHMENT 1:

Dissolved oxygen analysis with OptiOx optical sensor

1. Why do we have this method?

This method is used to measure dissolved oxygen in liquid samples.

2. When to use this method?

The method is typically used in chemical shelf life analysis of products. Oxygen in the head space of Pure-Pak cartons and dissolved oxygen in the product will aim for equilibrium. Dissolved O_2 decrease over time as it takes part in degradation reactions in the product, e.g. in degradation of ascorbic acid (vitamin C).

3. Who to use this method?

This method is to be used by personnel in Food & Material Science Department with required competence.

4. Principle

The InLab OptiOx optical dissolved oxygen instrument is a self contained portable instrument, which measures dissolved oxygen in liquid samples. The instrument records temperature and compensates for air pressure. It consists of an InLab OptiOx optical dissolved oxygen sensor connected to a Mettler Toledo SevenGo pro™ SG9 that has a display and is controlled by soft keys on the front of the instrument. In addition there is a calibration tube.

The InLab OptiOx optical dissolved oxygen sensor is based on Rugged Dissolved Oxygen (RDO) technology. The micro-optical technology incorporated in the RDO sensor centers around the methodology of Lifetime-based Luminescent Dissolved Oxygen detection. This solid-state method uses LEDs to excite a fluorescent material, while an optical receptor gauges the duration, or lifetime, of the event. The duration of fluorescence is inversely proportional to the ambient amount of dissolved oxygen in the water. Because the oxygen is not chemically consumed during the measurement, stirring is superfluous.

5. Equipment

- SevenGo pro™ SG9 with InLab OptiOx optical dissolved oxygen sensor from Mettler Toledo.
- Calibration tube

6. How to perform?

Under equilibrium conditions, the partial pressure of oxygen in air-saturated water is equal to the partial pressure of oxygen in water-saturated air. The OptiOx sensor calibrated in water-saturated air will correctly read the partial pressure of oxygen in water samples.

When measuring low concentration samples (< 1mg/L, 1 ppm), a second calibration with a zero oxygen standard may be done (Annex 1). It should, however, be taken into consideration that zero-point calibrations frequently are a source of error. Due to the very low zero current of the sensors, a zero-point calibration is unnecessary even for measurements at low oxygen concentrations.

6.1 Daily calibration procedure

The first point of a DO calibration is done in water-saturated air (100% O_2).

- Remove the calibration tube cap and remove the sponge
- Saturate the sponge with distilled water and squeeze the excess water out of the sponge
- Reassemble the calibration tube
- Make sure that no water droplets are on the surface of the sensor cap
- Slide the calibration tube over the front of the sensor until the calibration tube is firmly connected to the sensor
- Allow at least five minutes for the temperature to stabilize prior to calibration
- Press **CAL**
    - **Cal 1** appears on the display
    - The meter endpoints according to the preselected endpoint mode, automatically after the signal has stabilized or after pressing **READ**. The standard value is shown on the display.
- Press **End** to accept the calibration and return to sample measurement
    - The calibration result is shown on the display
- To reject the calibration, press **Exit**

6.2 Measurement of samples

- Place the sensor in the sample.
    - Make sure that both the DO sensor and the temperature sensor are immerged in the solution. (The temperature sensor is the small metal disc found approximately 3.5 cm from the sensor tip).
- Press READ to start a measurement
    - The endpoint format blinks, indicating a measurement in progress
    - As soon as the measurement is stable according to the selected stability criterion, the stability icon (/¯) appears.
- The measurement can be presented in different units (mg/L, ppm and %) by pressing MODE.

- An "A" presenting the endpoint format on the display, indicates that an "automatic endpoint" is selected. If an automatic endpoint is selected, the measurement stops as soon as the stability icon appears over the "A".
- If the "manual endpoint" format is selected, press READ to manually stop the measurement. The endpoint symbol will then appear over the "M".
- If the "timed endpoint" format is selected, press READ to manually stop the measurement. The endpoint symbol will then appear over the "T".

Preselected format	Start of measurement	Signal stability	Endpointed measurement [1]
Auto endpoint	A	/A	/A
	A	Read ⟹	/M
Manual endpoint	M	/	Read ⇨ /M
	M	Read ⟹	/M
Timed endpoint	T	/	🕒 ⇨ /T
	T	Read ⟹	/M

- The endpoint format can be changed by pressing **MENU** and select **Endpoint formats.**

6.3 Temperature compensation

If the temperature prove is connected and working, "ATC" (automatic temperature compensation) and the sample temperature are displayed. If "MTC" shows in the display, the temperature should be entered manually.
To set the temperature manually, press **MENU** and select **Temperature settings**.

The menu settings are further described in the Operating instructions.

TABLE 1: SENSOR SPECIFICATION LIMITS

	Measuring range	**Resolution**	**Accuracy**
Dissolved oxygen (mg/L, ppm)	0.00 – 50.00	0.01	± 0.1 mg/L from 0 – 8 mg/L ± 0.2 mg/L from 8 – 20 mg/L ± 10 % from 20 – 50 mg/L
Saturation (%)	0 – 500	0.1	
Temperature DO (°C)	0 – 50.0	0.1	± 0.1 °C
Pressure (mbar)	500 – 1100	1	± 1 mbar

6.4 Storage after use and general maintenance

(a) Meter

Never unscrew the two halves of the housing.

(b) OptiOx sensor

Cleaning

- Do not remove the sensor cap
- Rinse the sensor with distilled water daily after use
    - Gently wipe with a soft cloth if bio fouling is present.
    - If extensive mineral build-up is present, soak the cap in vinegar for 15 min
    - Soak the sensor in de-ionized water for 15 min and blot it dry with a lint-free tissue
    - After cleaning the sensor, a 1-point calibration should be performed
- Do not use organic solvents or soaps to clean the cap.

Storage

- Put the sensor in a beaker with distilled water or in the calibration tube, making sure to wet the sponge with distilled water
- Keep the sensor away from direct sunlight during storage
- Do not remove the sensor cap

Maintenance

The sensor cap for InLab® OptiOx oxygen sensors provides reliable results 365 days of the year. The meter will display a message "sensor cap expired" when the cap need to be replaced. For replacement, old caps can simply be removed and new ones fitted. The procedure is described in the Operating Instructions (p. 38).

Troubleshooting

TABLE 2: EXAMPLES OF TROUBLESHOOTING FOR THE SEVENGO PRO™ SG9 WITH INLAB OPTIOX OPTICAL DISSOLVED OXYGEN SENSOR.

Issue	Recommended Action
Unable to calibrate	Verify the calibration setup and procedure. Make sure that no water droplets are on the surface of the cap.
Measurements are unstable	Measurements may take longer if the solution temperature is unstable
Measurements is too low	Salt may be present in the sample. Set the salinity factor in the meter.
Wrong temperature displayed	Verify that the temperature sensor is immersed in the solution

- Rinse the sensor thoroughly in distilled water, blot it dry with a lint-free tissue and examine the cap for scratches or discoloration
- Remove the cap from the sensor and make sure that there is no water inside the cap, the optical window is clean and clear the O-rings are intact and have a thin coating of silicone grease and the spring contacts are undamaged
- If readings continue to be erratic and unstable, a replacement of the cap may be necessary.

Error messages are displayed next to the warning triangle and are described in the Operating Instructions.

7. Reporting of results

A minimum of three, preferably five, parallels should be done of each variable. The results from the dissolved oxygen analysis should be registered in FOR13020 Shelf Life Test Result Form and a report should be written (normally an overall report is written for a chemical and sensory shelf life test).
Statistical analysis where the results are tested for significance on 95% level (e.g. ANOVA General Linear Model (GLM)) should be done.

8. Specification

There is no specification of the dissolved oxygen content in products.

9. Validation

This method is not validated internally.

10. References

- Mettler Toledo SevenGo pro™/ OptiOx™ Operating Instructions
- FOR13020 Shelf Life Test Result Form

11. Revisions

This method has been revised because the Micro O_2 logger is replaced by the Mettler Toledo SevenGo pro™ SG9 instrument with InLab OptiOx optical dissolved oxygen sensor, which gives more stable readings without consumption of the product.

12. History

Created date: 2005-12-20 Created by: Elin M. Johansen
Revised date: 2011-12-14 Revised by: Elin M. Johansen

Created / modified	**Approved by / date:**
Date: 2013-09-17	Date: 2013-09-17
Name: Elin M. Johansen	Name: Jorunn Bjørnstad
Position: Laboratory engineer	Position: Manager Food & Material Laboratories
Sign:	Sign:

Responsible department:	Food & Material Sciences

13. Annex 1

Procedure for a 2-point calibration

The second point of a DO calibration is done with a zero oxygen solution.

- For the first point, follow the steps on how to make a 1-point calibration, but do not press **End**.
- After removing the sensor from the calibration tube and rinsing the sensor with de-ionized water, place the InLab OptiOx in a zero oxygen solution.
- Allow at least five minutes for the sensor to equilibrate prior to calibration.
- Press CAL
    - Cal 2 appears on the display
    - The meter endpoints according to the preselected endpoint mode, automatically after the signal has stabilized or after pressing **READ**. The relevant standard value is shown on the display.
- Press **End** to accept the calibration and return to sample measurement
    - The calibration result is shown on the display
- To reject the calibration, press **Exit**
- Thoroughly rinse the sensor under running water and blot it dry with a lint-free tissue

If the sensor is sluggish or inaccurate after a zero point calibration, not all of the zero oxygen was removed from the sensor. A very thorough soaking and rinsing of the sensor in distilled water is required to remove all of the zero oxygen solution and restore the sensor performance

ATTACHMENT 2:

Head space gas analysis of O_2 and CO_2 by using Check Mate II

1. Why do we have this method?

The purpose of the method is to analyze the concentration of O_2 and CO_2-gas in head space of Pure-Pak cartons.

2. When to use this method?

The method is typically used in:

- Chemical shelf life analysis of products.
- Analysis of effect of N_2-gas flushing of head space after filling in aseptic filling machines.
- Analysis of gas tightness in head space of cartons.
- Possible microbiological growth in product (production of CO_2).

Measuring range:	0 – 100%
Zirkonia sensor (O_2) accuracy:	$\pm$ 0.01% absolute in range below 1% $\pm$ 1% relative in range above 1%

Zirkonia sensor (O_2) resolution:	0.1% absolute in range above 10% 0.01% absolute in range above 1% 0.001% absolute in range below 1%
CO_2 sensor accuracy:	$\pm$ 0.5% absolute $\pm$ 1.5% of reading
CO_2 sensor resolution:	$\pm$ 0.1% absolute

3. Who to use this method?

This method is used by personnel in Food & Material Science Department with the required competence.

4. Principle

O2 (g) in head space and dissolved O_2 in the product will aim for equilibrium. Dissolved O_2 decrease over time as it takes part in degradation reactions in the product. This leads to a simultaneously decrease in head space O_2. Analyzing head space O_2 can therefore be used as a parameter in evaluation of chemical shelf life of a product over time and to evaluate gas tightness of cartons.
In aseptic cartons, the head space is normally flushed with N_2-gas directly after filling to a level below 5%. The effect of the N_2-gas flushing can be evaluated by analyzing the head space oxygen content.
If the product is contaminated by microorganisms, production of CO_2 might occur. Analyzing head space CO_2 can therefore be done as an initial test to investigate for microbial growth. This has to be confirmed by microbiological analysis of the product.

5. Equipment

The Check Mate II instrument has a Zirkonia based O_2 sensor and Non Dispersive Infrared CO_2 sensor. Both sensors are compensated for temperature- and pressure in the soft ware.

Sample volume:	6 ml
Sample time:	10 sec
Heating time (at start-up):	10 min
Operating conditions:	0 to +45^{o}C, less than 95% RH, non condensing.
Storage conditions:	-20 to + 60^{o}C, less than 95% RH, non condensing.

6. How to perform?

Check Mate II is based on an ‘all-in-one’ concept with a ‘state-of-the-art’ sensor technology. This means that the instrument perform self diagnostics and demonstrate normal user failures. No internal calibration is needed. An external calibration should be performed once a year by Nordic Supply AS.

1. Before starting with the analysis, it should be checked that the needle, tubing and filers are correctly attached to the instrument.

2. The instrument is normally set to ‘sleep mode’ when a test is finished. To start-up the instrument again, push a random button. The instrument will automatically start. A self test and heating is done. This takes approximately 10 minutes.

3. Perform a test measurement of the surrounding air. The result should be 20.9-21.0% O_2 and 0.04% CO_2.

4. Place a round septum on the top of the carton as shown in picture X.

5. Stick the needle into the septum and the carton. Note: held the needle in a horizontal position so that product is not sucked up and into the instrument.

6. Push the start button.

7. The result might be stored in the instrument, but is normally written down by the operator.

8. The instrument is normally set to ‘sleep mode’ in the menu when a test is finished.

9. The instrument is calibrated externally once a year. The plan for the next calibration could be found in the Journal of maintenance for the chemical laboratory.

7. Reporting of results

The results should be reported in FOR13020 Shelf life test result form. ANOVA analysis (e.g. General Linear Model) should be done on the results to evaluate possible significant differences on 95% level between the variables throughout the storage period.

The head space gas analysis of O_2 and CO_2 is normally a part of a chemical shelf life test, and the results are included in a final report together with the other results from the test.

8. Specification

For aseptic cartons flushed with N2-gas the concentration of head space O_2 should be less than 5%. For all cartons in a shelf life test, the concentration of head space O_2 should decrease over time, otherwise the carton might not be gas tight.

9. Validation

This method is not validated internally.

10. References

- Check Mate II Brugerguide 05/2009, PBI Dansensor

11. Revisions

This method has been revised because the Varian CP-4900 Micro-GC has been replaced by the check Mate II instrument. Check Mate Ii has been proven to give faster readings and the instrument needs less maintenance than the Micro-GC.

12. History

Created date: 2005-12-23 Created by: Anne Ebbesen
Revised date: 2011-12-14 Revised by: Elin M. Johansen

Created / modified	**Approved by / date:**
Date: 2013-09-16	Date: 2013-09-16
Name: Elin M. Johansen	Name: Jorunn Bjørnstad
Position: Laboratory engineer	Position: Manager Food & Material Laboratories
Sign:	Sign:

Responsible department:	Food & Material Sciences

ATTACHMENT 3:

VITAMIN C (ASCORBIC ACID) IN JUICES

1. Why do we have this method?

This method is used to analyze the content of vitamin C (in this method designated as ascorbic acid) in fruit juices. Vitamin C content is an important quality parameter in juices as it will be degraded in presence of oxygen. The process is dependent on temperature and time. Vitamin C analysis is an important analysis in chemical shelf life studies of fruit juices.

2. When to use this method?

This method is used to determine ascorbic acid content in single strength juices and reconstituted concentrates provided they do not contain ferrous Fe, stannous Sn, cuprous, sulfite or thiosulfate ions.

3. Who to use this method?

This method is to be used by personnel in Food & Material Science Department with required competence.

4. Principle

Ascorbic acid reduces oxidation-reduction indicator dye, 2,6-dichlorophenol-indophenol, to colorless solution. At endpoint, excess unreduced dye is rose ping in acid solution. Titration is performed in presence of meta-phosphoric acid solution to avoid auto-oxidation of ascorbic acid at high pH.

Linear Range[1]: 5 – 50 mg/100 ml

Detection limit: 5 mg/100 ml

Precision: 50 mg/100 ml, %RSD 0.11
25 mg/100 ml, %RSD 0.36
10 mg/100 ml, %RSD 0.96

Accuracy: 99%

1. Application book 716 DMS Titrino

5. Equipment

4 L glass beaker
2 L glass bottle
250 ml Erlenmeyer flask
150 ml glass beaker
500 ml (1000 ml) graduated flask
100 ml graduated flask
Glass funnel
Volumetric pipettes, 5 ml and 10 ml
Measuring equipment

Analytical balance
Magnetic stirrer
pH meter
Titrator, Metrohm 716 DMS Titrino equipped with 730 Sample Changer
Redox electrode, 6.0451.100 (Pt ring) or 6.0452.100 (Au ring)

The titrator and the keyboards connected to it and the sample changer are presented in Appendix 1.

6. Reagents

All reagents must be of analytical grade. Use only de-ionized water.

- meta-Phosphoric acid (Merck 1.00546.0500)
- Vitamin C (L(+)-ascorbic acid, Merck 1.00127)
- 2,6-Dichlorophenolindophenol sodium salt dihydrate (Merck 1.03028.0025)
- Potassium hydroxide pellets (Merck 1.05029.1000)
- De-ionized water

7. How to perform?

7.1 Preparation of reagents

(a) Acid solution

- Dissolve 60 g HPO_3 (meta-phosphoric acid) in 4 L distilled/ de-ionized water. Use magnetic stirring.
- Adjust pH to 3.5 - 4.0 with potassium hydroxide pellets. Add 1 tablespoonful at first, and then ad 1-2 pellets until you have the right pH.
- Transfer the solution to a closable container.

The solution remains satisfactory in refrigerator for 7-10 days

(b) Indophenol standard solution

> Hint:
> 500 ml indophenols standard solution is usually enough in most shelf-life studies (up to 12 samples). If more than 12 samples are to be tested, 1000 ml of the solution should be made

- Dissolve 0.500 g 2.6-Dichlorophenolindophenol Na-salt in approximately 100 ml de-ionized water in an Erlenmeyer flask.

> Hint:
> Take precautions against inhalation of the fine powder. Use protective gloves and safety goggles!

- Cover the Erlenmeyer flask with Alu-foil to prevent fumes and splashing, and heat the solution to about 60°C under magnetic stirring.
- Cool the solution to room temperature still covered with Alu-foil. The solution is light sensitive.
- Filter the solution through a black ribbon filter into a 500 ml volumetric flask. To ensure the most accurate concentration, use a spray bottle with de-ionized water to "wash" the filter.
- Make up to volume with de-ionized water. Transfer immediately to the amber glass bottle that is connected to the titrator.
- Replace the de-ionized water in the burette with the fresh solution by emptying the burette 3-4 times. Make sure that the burette tip is placed in a waste beaker. The burette is emptied by pressing down the "DOS" button on the titrator. Refill the burette by pressing "START/fill", and repeat the procedure. The DOS dispensing rate can be controlled by the turning the **dV/dt** knob on the titrator (10 is max).

Make a fresh solution each time, do not store. Empty the burette of indophenol solution after use. The solution can crystallize and damage the piston and glass and thus cause inaccurate. Fill the burette with de-ionized water.

> Hint:
> If crystallization is observed in the burette, it can be washed with ethanol.

(c) Standard ascorbic acid solution

Weigh exactly 50 mg (0.050 g) pure ascorbic acid and transfer quantitatively into a 100 ml volumetric flask. Dissolve and make up to volume with acid solution. Make the solution just before standardization. Do not store.

7.2 Preparations of the Metrohm 716 DMS Titrino titrator and the 730 Sample Changer

- Control that the canister contains enough de-ionized water for the automatic rinsing in between the samples.
- Place the burette tip in the correct position in the titration head.
- Control that the electrode contains enough electrolyte. The liquid level should reach to approx. 0.5 cm underneath the electrolyte fill hole.
- Rinse the electrode with de-ionized water and check the Pt- or Au- ring for oxidation.
- Rub the ring and diaphragm carefully with a wet soft tissue to remove potential residue.
- Place the electrode in the titration head.

7.3 Standardization of indophenol solution

- Transfer by using a full pipette 5 ml of ascorbic acid solution into a 150 ml beaker and add acid solution until the beaker contains approximately 140 ml liquid. Make sure that the liquid level is above the diaphragm on the electrode.
- Place a beaker with approx. 120 ml de-ionized water into rack position 16.
- Choose method "STANDARD" by pressing "user meth" on the key pad. ">recall method" appears in the display. Press "enter" and repeat pressing "select" until "STANDARD" appears in the display, then press "enter" again. The light for "statistics on" will appear.
- Titrate rapidly the ascorbic acid solution by pressing "START" on the SC controller keypad connected to the sample changer. Always run the sample in triplicate. The average of these three samples is the dye titer. Verify the number of samples registered in the sample changer by pressing "PARAM" until "number of samples: X" appears. (X is the number of samples, max 15). Change the number of samples by entering the correct number and "ENTER".

Calculation of dye titer:
This calculation is done automatically by the Titrino, since it's stored in the STANDARD method.

Dye titer (factor) = $T = CO_1/EP_1$

Where

$CO_1 = 2.5$ (mg ascorbic acid)
EP_1 = the number of ml of indophenol solution

Formula entered in saved titration method:
$RS1 = CO_1/EP_1$

Sample validation:
To ensure that the juice measurements give accurate values, it is recommended to test the instrument against a defined sample of ascorbic acid. An addition of 10 mL standard in a beaker should give 50 mg/100 mL ascorbic acid. It is recommended to test this sample together with the juice samples.

7.4 Ascorbic acid in juice

- Take out 10 ml sample by using a full pipette and transfer to a beaker. Add acid solution to approximately 120 ml. It is important that the liquid level is above the diaphragm on the electrode. Run two parallels from each carton.
- Choose method "Vit C" by pressing "user meth" on the key pad. ">recall method" appears in the display. Press "enter" and repeat pressing "select" until "Vit C" appears in the display, then press "enter" again. The light for "statistics on" will disappear.
- Press "C-fmla" and enter the dye titer result from the standardization (CO1). Press "enter" and "CO2: 10" will appear in the display. Press "enter" again or "QUIT" to exit.
- Verify the number of samples registered in the sample changer by pressing "PARAM" until "number of samples: X" appears. (X is the number of samples, max 15). Change the number of samples by entering the correct number and "ENTER".

Hint:
The titrator will automatically start with the sample in position 1. If the first sample is placed in another position, the start position can be entered manually by pressing "SAMPLE" or "7" on the SC controller keypad, then enter the position of the first sample. Confirm by pressing ENTER.

- Titrate immediately with indophenol solution by pressing "START" on the on the SC controller keypad connected to the sample changer.

Hint:
Prior to titration of samples with high ascorbic acid concentrations, the start volume can be altered, in order to save time. This is done by pressing "parameters" on the DMS Titrino Keyboard and pressing "enter" until "start V" appears for the second time. The desired start volume is entered and confirmed by pressing "enter". Press "QUIT" to exit.

Calculation, Content of ascorbic acid in mg/100 ml juice

The ascorbic acid content is calculated by the Titrino when the formula is stored in the method.

mg ascorbic acid/ 100 ml juice = $T * EP_1 * 10$

Where

T = Mean dye titer from standardization
EP_1 = the number of ml of indophenol solution

Formula entered in the saved titration method:
$RS1 = CO_1 \cdot EP_1 \cdot CO_2$

Where

CO_1 = Mean dye titer from standardization
$CO_2 = 10$
EP_1 = the number of ml of indophenol solution

8. Reporting of results

A minimum of three, preferably five, parallels should be done of each variable. The results from the ascorbic acid analysis should be registered in FOR13020 Shelf Life Test Result Form and a report should be written (normally an overall report is written for a chemical and sensory shelf life test).
Statistical analysis where the results are tested for significance on 95% level (e.g. ANOVA General Linear Model (GLM)) should be done.

9. Specification

There is no limit for the vitamin C/ascorbic acid content in fruit juices according to Commission Directive 2009/106/EC, but many customers have declared their own limit on the cartons.

10. Validation

This method is not validated internally.

11. References

1. Application book 716 DMS Titrino
2. Vitamin C in Vitamin Preparations and Juices, AOAC 967.21
3. Fruits, vegetables and derived products – Determination of ascorbic acid content, ISO 6557/2-1984(E)
4. Determination of L-Ascorbic acid, IFU Method 17
5. Commission Directive 2009/106/EC of 14 August 2009 amending Council Directive 2001/112/EC relating to fruit juices and certain similar products intended for human consumption.
6. FOR13020 Shelf Life Test Result Form

12. Revisions

This method has been revised to better explain the performance of Metrohm 716 DMS Titrino.

13. History

Created date: 2002-07-26 Created by: Anne Ebbesen
Revised date: 2011-12-14 Revised by: Elin M. Johansen

Created / modified	**Approved by / date:**
Date: 2011-12-14	Date:
Name: Elin M. Johansen	Name:
Position: Laboratory engineer	Position:

Sign:	Sign:

Responsible department:	Food & Material Sciences

Appendices

1. Titrator, Methrom 716 DMS Titrino set up

The settings presented below are stored in the respective methods. They can be reentered by following the procedure described in the instruction manual p. 6, but choosing mode "MET" instead of "DET", and "Ipol" instead of "pH", as presented in the example. It is not necessary to perform the test titration described in the procedure, nor entering sample size. The report settings are "curve; full".

ATTACHMENT 4:

Table X: **Not measured by me, brix not the same as in thesis**

Analysis	Value in concentrate	Unit	Value in 11,18 brix diluted concentrate	Unit
Titrable acid (pH 7 tart.a) (IFU Nr. 3)	55.63	g/kg	9.44	g/l
Titrable acid (pH 8,1 cit.a.) (IFU Nr. 3)	49.32	g/kg	8.37	g/l
Total acid pH 8,1 (IFU No. 3)	770.6	meq/kg	130.8	meq/l
L-malic acid (IFU No. 21)	14.98	g/kg	2.54	g/l
Citric acid (IFU No. 22)	49.01	g/kg	8.32	g/l
Iso-citric acid (IFU No. 54)	399	mg/kg	68	mg/l
Ascorbic acid (IFU No. 17a)	2172	mg/kg	369	mg/l
Sucrose (IFU No. 56)	268.3	g/kg	45.5	g/l

Glucose (IFU No. 55)	111	g/kg	18.8	g/l
Fructose (IFU No. 55)	121.1	g/kg	20.6	g/l
Potassium (IFU No. 33)	12.04	g/kg	2.04	g/l
Sodium (IFU No. 33)	98	mg/kg	17	mg/l
Calcium (IFU No. 33)	604	mg/kg	103	mg/l
Magnesium (IFU No. 33)	625	mg/l	106	mg/l
Phosphate (IFU No. 35)	3361	mg/kg	571	mg/l
Total phosphorous (P) (IFU No. 35)	1098	mg/kg	186	mg/l
Nitrate (IFU No. 48)	3	mg/kg	traces	
Proline (IFU No. 49)	3945	mg/kg	670	mg/l

ATTACHMENT 5:

Table 8: Balanced ANOVA-analysis, 8*5 different EVOH samples stored for 8 weeks. ANOVA was conducted to examine the effect of the type of barrier imperfection on the **vitamin C** content in juice over 8 weeks, as well as the interaction effect of the type of barrier imperfection and storage time.

Source	DF	SS	MS	F	P
Type of barrier imperfection (BI)	7	224,59	32,08	113,88	0,00
Week no. (WN)	4	5829,04	1457,26	5172,17	0,00
BI*WN	28	293,99	10,50	37,27	0,00
Error	160	45,08	0,28		
Total	199	6392,70			

S: 0,53 (Model description of response) R^2: 99,12% (Variation in response explained by the model)

Table 9: Balanced ANOVA-analysis, 8*5 different EVOH samples stored for 8 weeks. ANOVA was conducted to examine the effect of the type of barrier imperfection on the **dissolved O_2** content in juice over 8 weeks, as well as the interaction effect of the type of barrier imperfection and storage time.

Source	DF	SS	MS	F	P
Type of barrier imperfection (BI)	7	12,53	1,79	13,13	0,00
Week no. (WN)	4	50,16	12,54	91,92	0,00
BI*WN	28	12,76	0,46	3,43	0,00
Error	160	21,83	0,14		
Total	199	97,28			

S: 0,37 R^2: 72,09%

Table 10: Balanced ANOVA-analysis, 8*5 different EVOH samples stored for 8 weeks. ANOVA was conducted to examine the effect of the type of barrier imperfection on the **headspace Oxygen concentration** in a juice carton over 8 weeks, as well as the interaction effect of the type of barrier imperfection and storage time.

Source	DF	SS	MS	F	P
Type of barrier imperfection (BI)	7	814,80	116,40	84,08	0,00
Week no. (WN)	4	108,03	27,01	19,51	0,00
BI*WN	28	332,09	11,86	8,57	0,00
Error	160	221,50	1,38		
Total	199	1476,43			

S: 1,17 R^2: 81,34%

Table 11: Balanced ANOVA-analysis. 2*5 aluminum and EVOH barrier cartons. ANOVA conducted to find possible material effect on **vitamin C** content in in juice stored in gable top cartons with **no barrier imperfections**, over 8 weeks storage.

Source	DF	SS	MS	F	P
Type of barrier imperfection (BI)	1	118,58	118,58	504,09	0,00
Week no. (WN)	4	806,45	201,61	857,07	0,00
BI*WN	4	9,50	2,37	10,09	0,00
Error	40	9,41	0,24		
Total	49	943,93			

S: 0,49 R^2: 98,78%

Table 12: Balanced ANOVA-analysis. 2*5 aluminum and EVOH barrier cartons. ANOVA conducted to find possible material effect on **headspace oxygen concentration** in gable top cartons with **no barrier imperfections**, over 8 weeks storage.

Source	DF	SS	MS	F	P
Type of barrier imperfection (BI)	1	56,47	56,47	140,72	0,00
Week no. (WN)	4	562,69	140,67	350,56	0,00
BI*WN	4	12,01	3,00	7,48	0,00
Error	40	16,51	0,40		
Total	49	647,22			

S: 0,63 R^2: 96,96%

Table 11: Balanced ANOVA-analysis. 2*5 aluminum and EVOH barrier cartons. ANOVA conducted to find possible material effect on **vitamin C** content in in juice stored in gable top cartons with **500 µm holes in gas barrier**, over 8 weeks storage.

Source	DF	SS	MS	F	P
Type of barrier imperfection (BI)	1	5,54	5,54	15,93	0,00
Week no. (WN)	4	1284,00	321,00	923,22	0,00
BI*WN	4	9,41	2,35	6,77	0,00
Error	40	13,91	0,35		
Total	49	1312,86			

S: 0,59 R^2: 98,70%

Table 12: Balanced ANOVA-analysis. 2*5 aluminum and EVOH barrier cartons. ANOVA conducted to find possible material effect on **headspace oxygen concentration** in gable top cartons with **500 µm holes in gas barrier**, over 8 weeks storage.

Source	DF	SS	MS	F	P
Type of barrier imperfection (BI)	1	4,57	4,57	5,64	0,00
Week no. (WN)	4	8,24	2,06	2,54	0,00
BI*WN	4	5,16	1,29	1,59	0,00
Error	40	32,40	0,81		
Total	49	50,36			

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