Integration of transparency into packaging design

Emma Pignères

DIVISION OF PACKAGING LOGISTICS | DEPARTMENT OF DESIGN SCIENCES
FACULTY OF ENGINEERING LTH | LUND UNIVERSITY

2018

MASTER THESIS

FIPDes

Food Innovation & Product Design

This Master's thesis has been don within the Joint European Master Course FIPDes, Food Innovation and Product Design.

Integration of transparency into packaging design

Impact of light exposure on fermented products

Interaction pack/product: barriers needed to protect product's quality

Prevention of photo oxidation through packaging design

How does light in supermarket affect the product during storage?

Emma Pignères

LUND UNIVERSITY

Impact of light exposure on fermented milks and fermented juices

Copyright © 2018 Emma Pignères

Published by
Division of Packaging Logistics
Department of Design Sciences
Faculty of Engineering LTH, Lund University
P.O. Box 118, SE-221 00 Lund, Sweden

Subject: Food Packaging Design (MTTM01)
Division: Packaging Logistics
Supervisor: Daniel Hellström
Examiner: Henrik Pålsson

This Master´s thesis has been done within the Joint European Master Course FIPDes, Food Innovation and Product Design.

www.fipdes.eu

ISBN 978-91-7753-755-7

Abstract

Transparency has become a key sales pitch for companies! It enables them to show their product and tell the consumers they do not have nothing to hide. But from a technical point of view, transparency implies many risks for product's quality such as photooxidation and other reactions leading to colour changes, off-flavours and decrease of product's quality. Therefore, the objective of this master thesis, conducted within the French company Danone, was to study the impact of transparency on the sensitive molecules present in fermented animal and vegetal milks and fermented cow milk mixed with fruit preparations. The final aim was to propose packaging solutions and to provide design guidelines to integrate transparency into Danone's packaging. First of all, the current light test protocol used in Danone was assessed, and a new one was defined. Part of this new light test protocol was then applied on four fermented products: a fermented cow milk, a fermented almond-based milk, a fermented cow milk mixed with 40% of red fruits preparation and a fermented cow milk mixed with 40% of green fruits preparation. Several packaging options were studied on these products. For the two first products, gathered in Case 1, PP + EVOH and PP + EVOH + UV block were tested and for the two last ones, Case 2, HDPE + UV block and PP + EVOH + UV block were used. Therefore, UV and visible light impact were more focused on for the first case, whereas oxygen barrier effect was more looked at in the second case. To follow-up the impact of light and oxygen on these four products, an organoleptic evaluation, a gas chromatography, pH and Dornic degree, colour and headspace gas composition measurements were set up.

Keywords: light exposure, photo-oxidation, packaging, packaging barrier, fermented products

Acknowledgments (or Preface)

This master thesis has been twenty weeks of great discovery of a new company environment, as well as scientific protocols and ways of thinking, and all of this would not have been possible without the help and support of many people I would like to thank here.

I would like to express my deep gratitude to Frédéric Chivrac, my supervisor in Danone, thanks to whom I learnt a lot during my thesis and who took a lot of time to support my work and make me discover the diversity of the packaging world!

Special thanks should be given to the whole global packaging team who hosted me in Danone, as well as all the people from other teams who helped me a lot during my project.

I would like to thank Daniel Hellström, who was my supervisor from Lund University during this master thesis and spent time to give me relevant and helpful feedbacks all along my thesis and enabled me to organise my thought in a better way, to Henrik Pålsson for being my examiner and to Erik Andersson who was always a good support. Assistance provided by Cilla Perlagen all along the year was also very valuable.

Finally, I would like to express my great gratitude to all FIPDes staff, teachers and students, for teaching me so many things during these two years, on both professional and personal aspects.

Lund, June 2018

Emma Pignères

Table of contents

List of figures

List of tables

List of graphs

List of pictures

List of acronyms and abbreviations

CCT	Correlated Colour Temperature
EVOH	Ethylene vinyl alcohol
FMOT	First Moment of Truth
HDPE	High-density polyethylene
OTR	Oxygen Transmission Rate
PET	Polyethylene terephthalate
PP	Polyethylene
SMOT	Second Moment of Truth
UV	Ultra Violet

1 Introduction

1.1 Background

More and more consumers want to see the products they are buying. In a study made by Simmonds *et al* (2018), more than half of consumers interviewed believed that it is important to be able to see the product through transparent packaging, and many companies now integrate it to their packaging. These products are evaluated as more trust-worthy. However, the product inside must be attractive and close to the consumers' expectation, otherwise the transparency will have a negative effect. Only a product which seems to be tasty, fresh and seems to have a superior quality will perform better in the market place within transparent packaging (Simmonds, Woods, & Spencer, 2018). This will affect the First Moment of Truth (FMOT), defined as the moment when the consumer sees the product, when he or she will decide to buy it or not for the first time. This must be considered if sleeves or anti-ultra violet (UV) filters are added: this may change the perception of the product if it impacts the colours of the pack and therefore of the product. But it must be considered also regarding the product itself and its evolution, which may be noticed by consumers through a transparent packaging and influence his or her intent of purchase or repurchase. This will affect the Second Moment of Truth (SMOT). Another way to evaluate the impact of transparency on the consumers' perception is to use Kano's theory. Professor Kano has defined five categories of perceived qualities: attractive quality, one-dimensional quality, must-be quality, indifferent quality and reverse quality (Löfgren & Witell, 2005). Transparency when it is well managed, i.e. when the product inside looks tasty and is not damaged by light exposure, is an attractive quality: this is a surprise and delight attribute which provides satisfaction when achieved fully, but consumers may not have been dissatisfied if the packaging was opaque. However, if the product inside does not look good or if light has a negative impact on product or packaging, transparency may become a reverse quality, which refers to a high degree of achievement resulting in dissatisfaction (Löfgren & Witell, 2005). Picture 1 is an example of transparency as a reverse quality during SMOT: indeed, the consumer can see after consuming all the product that stays in the pack and which he or she cannot consume.

Picture 1: Empty transparent packaging for dairy product with fruit preparation products – an example of reverse impact of transparency on consumer perception during SMOT

Transparency also means light penetration into the packaging and many food products are sensitive to light, which can be responsible for molecules' oxidation. One needs to keep in mind that light can activate the oxygen in the headspace of the packaging, which provokes the degradation of fatty acids, amino-acids, vitamins and proteins (Frederiksen, Haugaard, Poll, & Becker, 2003). Solutions to protect the product exist, such as nitrogen in the headspace to prevent these nutritional and organoleptic changes. However, if nitrogen is injected, the anaerobic microorganisms present in fermented milk can keep growing and trigger post-acidification of the fermented product. This is why impact of light and oxygen need to be assessed when a transparent packaging is chosen.

1.2 Research problem and question

Companies are more and more consumer-centred. Many studies have shown that consumers are looking for naturality. Transparent packaging is a way to bring naturality and trust-worthiness. However, many researches were done on light impact on products, such as milk and yogurts. It is well-known that UV wavelengths affect riboflavin (Bekbölet, 1990) in milk, and are therefore responsible for off-taste development. However, less studies have been done on the impact of visible light only on these products, and even less have been done on other products, such as plant-based products and dairy products when they are mixed with fruit and vegetable preparations.

To fill this gap, this thesis focused on both the impact of UV and visible light on three types of fermented products: fermented cow milk, fermented almond milk and a mix of fermented milk with fruits and vegetables preparations. The overall research question of this thesis is: how do UV and visible light, coupled with oxygen, affect product's organoleptic properties and main characteristics (colour, pH, bacteria count…), and therefore which level of protection is needed when transparency is integrated into their packaging?

1.3 Purpose and Goals

The purpose of this master thesis is to get more understanding of the impact of light and oxygen on three types of fermented products: cow milk, almond milk and cow milk mixed with fruit and vegetable preparation. To reach this purpose, three main goals were defined:
- to review the current light test protocol used in Danone and to define a new one to better evaluate the impact of light and oxygen on products.
- to understand the impact of light and oxygen on the products and the many reactions they can involve.
- to define for each kind of product studied the barriers needed into the packaging to protect the product. The final aim of the study is to provide packaging design guidelines for all these products.

1.4 Focus and demarcations

Five focuses were done: on the company, the packaging role which was studied, the kind of light, the temperature, and the type of packaging materials. First, this thesis was done within Danone company, a French multi-national company proposing food products within four categories: waters, essential dairy and plant-based products, medical nutrition and infantile nutrition. The thesis was conducted in Danone Research Centre in Palaiseau, in Essential dairy and plant-based products department. Therefore, a focus was not only done regarding the company, but also on the products, which were only fermented products. This thesis focuses on one aspect of the protection role of packaging: the protection against light and oxygen. Regarding the kind of light which will be focused on, fluorescent light and LED are the most used in supermarkets today, with an increasing enthusiasm towards LED technology: LED lighting in open dairy retail cases increased from about 15% in 2010 to almost 40% in 2014 (Johnson, et al., 2015). However, only fluorescent lights were available in the company, and this type of light stays majoritarian today in supermarkets. Photo-oxidation is also dependent on external conditions such as temperature. Fridges usually have a temperature of 4°C. The tests were done at this temperature. Finally, only plastic materials were studied, since they were the most suitable materials for the chosen products.

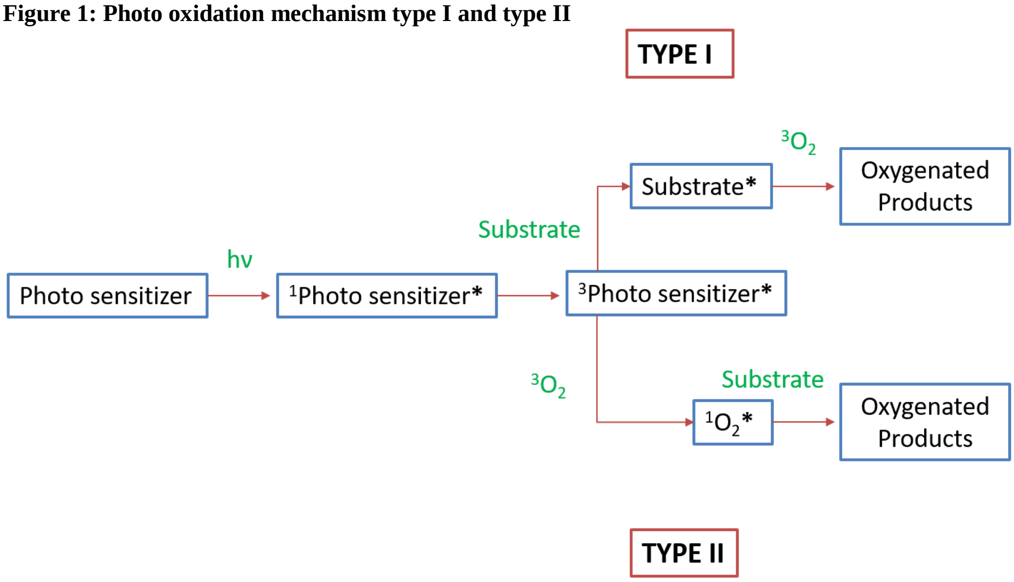
2 Literature review

Photo-oxidation mechanism was first explained, to understand the relation between light, packaging and product. In a second phase, sensitive components, such as photo-sensitizers or oxidable molecules, in dairy products, plant-based milks and fruits and vegetables were described. Then, protocols evaluating the impact of light on such products were analysed, in order to understand the optimal parameters and best tests for analysing light exposure effect on products. Finally, packaging properties were described, as their characteristics will be key to protect the product from photo-oxidation, based on the results from the light test.

2.1 Photo-oxidation mechanism

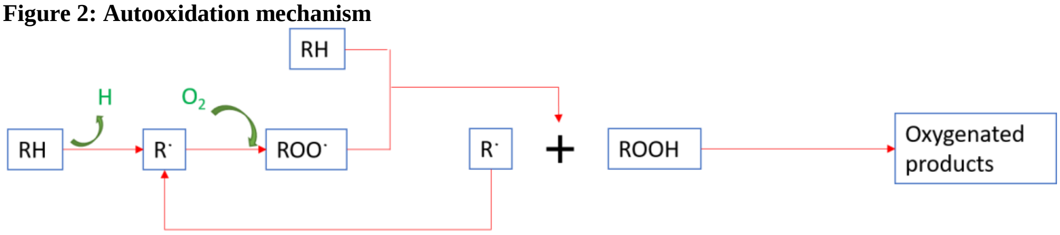
Oxidation can occur through two main reactions: autooxidation and photo-oxidation (Frankel, 1980). Photo-oxidation refers to oxidation reactions enhanced by light. Light is composed of several wavelengths, which can be absorbed by certain molecules, called photo sensitizers. These molecules are first excited by light and then trigger a cascade of photochemical reactions which may be direct, via isomerisation or rearrangements or indirect by energy transfer to other molecules, such as molecular oxygen or products of the photo-oxidation. As shown in Figure 1 the first phenomenon refers to type I mechanism and the second one refers to type II mechanism. (Borle, Sieber, & Bosset J.O., 2011). Type I oxidation occurs at low oxygen concentration and type II at high oxygen concentration (Decker, Elias, & McClements, 2010). This is why, even when products exposed to light have very little access to oxygen, photo-oxidation can still be observed, due to type I reactions (Intawiwat N. , 2011). In food, most of the sensitive molecules do not absorb light, but they are sensitive to the excited forms of oxygen. They are called **substrates** of photo-oxidation. Other compounds, such as riboflavin, hematoporphyrin, chlorophylls and protoporphyrin in dairy products, and other pigments in fruits and vegetable preparations have conjugated double bounds which enable them to absorb wavelengths in visible and UV spectrum. Therefore, they act as **photo sensitizers** (Intawiwat, et al., 2009).

Figure 1: Photo oxidation mechanism type I and type II



Besides photo-oxidation, another mechanism can trigger lipid oxidation, called autooxidation. This is a free radical chain reaction where unsaturated fatty acids (RH on Figure 2) loose hydrogen atom and create a free radical (R˙ on Figure 2) which reacts with oxygen to form peroxyl radicals (ROO˙ on Figure 2). Finally, peroxyl radicals react with a new unsaturated fatty acid to form hydroperoxides (ROOH on Figure 2) and secondary products which cause off-flavours. (Intawiwat N. , 2011). This reaction happens in presence of oxygen and unsaturated lipids, but is not enhanced by light.

Figure 2: Autooxidation mechanism



2.2 Sensitive components in milk and fruits preparations

Fermented milks, animal or plant-based, as well as fruit and vegetable preparations, contain sensitive components, which are likely to react with oxygen or most often with excited forms of oxygen obtained when exposed to light (Robertson, 2010). Some of them, such as proteins and lipids are common to all the products, the risk depending on their amount. But others, such as riboflavin in dairy products and betanin responsible for the colour in red vegetables for instance, are specific to certain kinds of products. Therefore, it is important to know very well the exact composition of each product before evaluating their light sensitivity in a laboratory.

2.2.1 **In fermented animal milk**

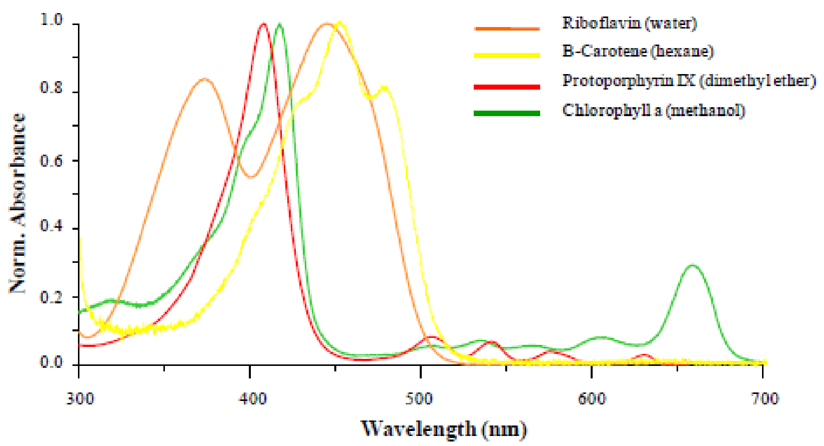
Sensitive components in animal milk are proteins, lipids, mainly unsaturated fatty acids, vitamins, such as riboflavin, and pigments such as β-carotene, chlorophyll and porphyrin compounds.

Lipids, mainly unsaturated fatty acids such as phospholipids, are the main compounds in food which are oxidised. (Sattar & Deman, 1975). Short chain aldehydes (pentanal, hexanal, heptanal and octanal), alkanals, alkenals, akladienals, alkanones and alkanols are produced and are responsible for oxidized flavours such as paper, cardboard, metallic, tallow and oily off-notes. (Mestdagh, 2005).

Among proteins, mainly whey proteins and side chain of 6 amino acids residues (tryptophan, tyrosin, phenylalanine, histidine, cysteine, methionine) can be oxidised (Sattar & Deman, 1975) . Major photo-products are flavourful sulfuric compounds such as dimethyl sulfide, disulphide, trisulphide, methaenethiol, methional and methyol mercaptans. They provoke activated flavours such as burnt, scorched, cabbage and mushroom flavours. (Decker, Elias, & McClements, 2010). Proteins produce off-flavour compounds faster than lipids, but activated flavours from proteins dissipate within a few days and are replaced then by oxidized flavours from lipids (Decker, Elias, & McClements, 2010).

Moreover, five photo-sensitizers, with conjugated double bounds or tetrapyrrole rings, have been described in dairy products. They are activated by UV and visible light, and their excited form will enhance photo-oxidation of other molecules such as lipids and proteins. Riboflavin is a photo-sensitizer, which absorbs mainly in UV wavelengths. Its degradation can be affected by lumichrome and carboxymethylflavin. Finally, riboflavin was found to be less stable at lower pH, which means that it is less stable in yoghurt which is fermented until pH reaches 4, than in milk for instance. (Sheraz, 2014). Other photo-sensitizers are more sensitive to visible light. Protoporphyrin IX, hematoporphyrin and chlorophyllic compounds are pigments which can react with light and provoke photo-oxidation of the product (Larsen, Geiner Tellefsen, & Dahl, 2009), (Intawiwat, et al., 2009). Even though chlorophyll a and b, hematoporphyrin and protoporphyrin IX are in lower amount than riboflavin, they have showed higher correlations to sensory properties compared to riboflavin (Wold, et al., 2005). Graph 1 shows the peaks of absorbance of the photo-sensitizers and pigments described above (Intawiwat N. , 2011). It also highlights that blue light and red light are the most harmful for dairy, whereas green light has been shown to cause less adverse effects regarding photooxidation.

Graph 1: Absorption normalized spectra for riboflavin in water, β-carotene in hexane, protoporphyrin IX in dimethyl ether and chlorophyll-a in methanol



Photosensitized oxidation can be prevented by other compounds in dairy products, such as β-carotene, ascorbic acid and α-tocopherol, which react rapidly with singlet oxygen. Moreover, β-carotene absorbs light in the same area as riboflavin (see Graph 1), and therefore protects against the degradation of this last molecule (Intawiwat N. , 2011).

2.2.2 **In fermented plant milk**

Lipids oxidation is the main phenomenon which can occur on these products.

Almond milk has the following lipid composition: 70% oleic acid, 20% linoleic acid and 8% saturated fatty acids, mostly in the form of palmitic acid. They are sensitive to oxygen and light and can trigger discoloration and rancidity of the drink. Linolenic acid is the first fatty acid oxidized and the products generated may trigger the lipoxidation of more highly saturated linoleic and oleic acids. Higher lipoxidation occurs under light: peroxides are generated and converted into carbonyls during the more advanced stages of reactions. Light was found to enhance the rates of hexanal production and those of 3-methylbutanal (Tazi, Plantevin, Di Falco, Puigserver, & Ajandouz, 2009).

2.2.3 **In fruit and vegetable preparations**

Vitamins and pigments are the most sensitive to light components in fruits and vegetables.

Vitamins A, B and C contained in fruit and vegetable preparation, are very sensitive to oxygen and light. They can be degraded by light-enhanced reactions and therefore trigger a nutritional loss of the product. Moreover, if protons are releases during these oxidation reactions, it can also provoke an acidification of the product, becoming sourer. Vitamin C for instance can give two hydrogen atoms to neutralise an oxidant molecule, via a redox reaction, followed by its irreversible transformation into dehydroascorbate. This reaction was shown to be enhanced by light (Lavoie, Chessex, Rouleau, Migneault, & Comte, 2004). However, vitamin C was shown to be more stable in fermented juices than in unfermented ones and fermented juices have higher amounts of antioxidants, such as polyphenols (Profir & Vizireanu, 2013). Furthermore, some vegetables, such as carrots, contain Vitamin E which can play an antioxidant role (Mauro, Guergoletto, & Garcia, 2016).

Carotenoids, present in many fruits and vegetables like apricot and carrots, have shown to be very sensitive to oxygen combined with UV-light: faster degradation of carotenoids was observed in these specific conditions (Christophersen, Bertelsen, Andersen, Knuthsen, & Skibsted, 1992). Other parameters which light can indirectly affects, have an influence on pigments stability: no degradation of carotenoids was observed after 8 days of storage at 4°C at pH lower than 3,15 (Bell, Alamzad, & Graf, 2016). Red beet pigments, such as betalains, were found to be more stable in presence of nitrogen and when stored in the dark: indeed, exposition to light accelerates pigment discolouration and darkening, even more at low pH. (Elbandy & Abdelfadeil, 2008). Light can thus affect product evolution, and the packaging can be influenced as well: β-carotene and betalanin, responsible for the colour respectively in carrots and red beets, can cause product discoloration and coloration of the bottle via plastic migration (Klewicka, 2010). This has an impact on the SMOT for consumers. A third well-known family of pigments, found in red fruits, is anthocyanin. This pigment’s stability is also affected by process, time and storage conditions. Anthocyanins, like carotenoids, have shown to be more stable at low temperature and at low pH such as 2,5 (Hornedo-Ortega, Alvarez-Fernandez, Cerezo, Troncoso, & Garcia-Parilla, 2016). However, these pigments are not sensitive to light exposure. They can even play the role of anti-oxidants in photo-oxidation reactions. Flavonoids, such as anthocyanins, have the capacity to inhibit and reduce the lesions caused by free radicals (Borari Lima, Duarte Correa, Aparecida Saczk, Pereira Martins, & Oliveira Castilho, 2003).

2.3 Light Test Protocols

Fourteen studies on photo-oxidation impact on dairy products were looked at in the literature. In all these protocols, six parameters were summed up in Table 1: type of product, type of lighting, power of the light, illumination, temperature and duration of the experimentation. Types of tests and analyses which were done were also noted and compared between themselves.

Table 1: Comparison of the light exposure tests found in the literature

Source	Product	Type of light	Power (Watt)	Illumination (Lux)	Temperature	Time
Danone	Dairy products	Fluorescent light	30-35	1500-2000	6±2°C	5d – 14h light and 10h dark
Duncan (2001)	Milk	Fluorescent light	40	1100-1300	4°C	d0, 7, 14 and 18 – 24h light
C.Papachristou (2006)	Pasteurised milk	Cool white fluorescent light	55	825±25	4±0,5°C	1d - 24h light
J.P. Wold (2006)	Cheese	Standard Fluorescent tube	58	5800	6°C (about)	Between 1 and 48h of light
H. Potts (2016)	Milk	LED & Fluorescent light		936±136 (LED) 1447±1072 (Fluo)	4°C	8 hours (LED) / 4hours(Fluo)
Borthersen (2016)	Milk	LED & Fluorescent light	/	4000 (LED) 2200(Fluorescent)	4°C	24h
Domingos (2014)	Yogurt	Fluorescent light	15	1000	5°C	35 days
Strand (2003)	Yogurt	Fluorescent light	18	3500	4°C	5 weeks
Becker (2003)	Yogurt	Fluorescent light	18	3500	4°C	5 weeks
Koyuncu & Tuncturk (2017)	Butter	White fluroescent bulbs	20	340-420	4°C	90 days
Larsen et al (2009)	Sour cream	Fluorescent light tubes	58	5610	4°C	36h
Marleen van Aardt (2000)	Milk	Fluorescent light		1100-1300	4°C	18 days
Johnson et al (2015)	Milk	Fluorescent light bulbs	32	2186 (range from 396 to 3970)	2,7°C	5 weeks
Zardin et al (2016)	Milk	Fluorescent tubes	15	10,6 W/m2	4,5±0,5 °C	1st experiment : 20H 2ndexperiment : 48h
Mestdag (2005)	Milk	Fluorescent tubes	36	2500	Ambient	

Regarding lighting, most studies use fluorescent tubes, with a very variable illumination, going from 825 lux to 5800 lux. Two studies out of fourteen used LED light, as a comparison with fluorescent light. Both studies showed that LED lamps, since they do not emit in UV and produce less heat, are less harmful for dairy products: milk exposed under LED and packed in high-density polyethylene

(HDPE) or polyethylene terephthalate (PET) showed less off-taste than the one exposed under fluorescent light (Potts, Amin, & Duncan, 2016).

Regarding power, one can notice that studies on yogurt generally use lamps with 15 to 18 watts and studies on milk are closer to 40 to 55 watts. Temperature was found to be 4 to 6 °C in all studies.

Moreover, Koyuncu and Tuncturk showed in their study that fluorescent illumination covers a large spectre over the shelves, meaning that all products do not receive the same amount of light over a shelf (Koyuncu & Tuncturk, 2017). Furthermore, depending on the position of the sample, one can be hidden by another one and thus be protected from light. Therefore, in a protocol, in order to compare samples which received exactly the same amount of light and remove these biases, two solutions exist : one can place the samples 20 cm apart from each other (Bothersen, McMahon, Legako, & Martini, 2016), or samples can be randomly interchanged to minimise unequal temperature fluctuations and light conditions (Becker, Christensen, Frederiksen, & Haugaard, 2003).

Duration of exposure was very different from one paper to another, going from 4 hours to 5 weeks. This has to be related to the real time products spent on supermarket shelves, depending on the country, the size of supermarket, its location, and on the product itself.

Finally, all the protocols found used a sensory test coupled with an analytical measure to follow product's oxidation, via front face fluorescence (Larsen, Geiner Tellefsen, & Dahl, 2009), mass spectrometry (Zardin, Silock, Siefarth, & Bremer, 2016) or Thiobarbituric Acid Reactive Substance Analysis (TBARS) (Johnson, et al., 2015). Sensory tests were done either by triangular test, to identify if participants could make the difference between the samples exposed to light and the ones stored in the dark (Johnson, et al., 2015), or by rating samples regarding their off-notes and off-tastes (Mestdagh, 2005) . In some literature, other tests were added to follow the impact of light on the product, such as bacteria count, determination of vitamins or headspace oxygen measurement (Papachristou, et al., 2006).

To conclude, literature provides many light test protocols to evaluate the impact of light on food products. One important learning from all of them is that parameters such as temperature and illumination are difficult to control over a shelf and solutions, such as random interchange during the test or very controlled environment, need to be found so every product receive the same amount of light and can be compared. Furthermore, having LED and fluorescent lighting, which both exist in supermarkets, is a good opportunity to compare their impact on the product. Moreover, analytical tests need to be set up since they help to understand which reactions have occurred in the products and can be thus correlated with sensory tests. Finally, all protocols need to be adapted to each country and type of product, regarding illumination, temperature and time of exposure.

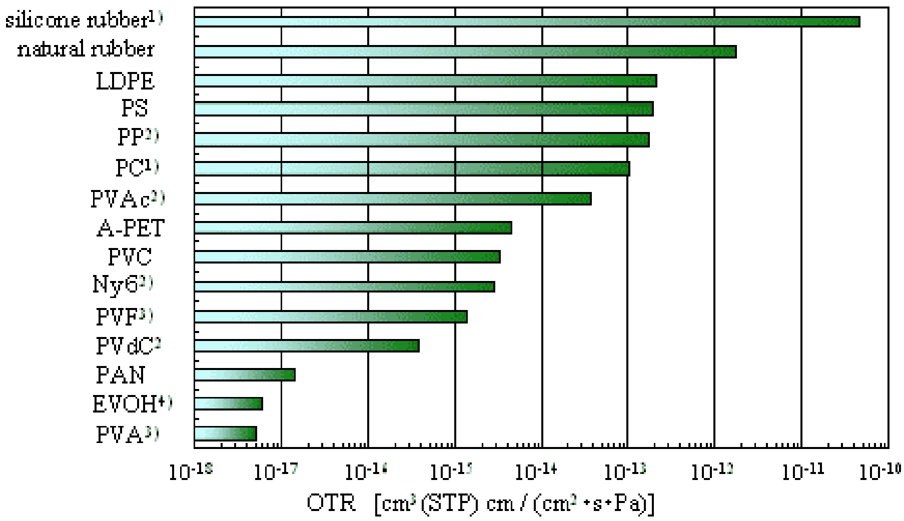
2.4 Proprieties of packaging materials

Lockamy described six main functions for packaging: protection, containment, apportionment, unitisation, communication and convenience (Lockamy, 1995). This master thesis focuses on the first one: protection. It must protect its content from outside environmental effects and protect the environment from the product. Deteriorative reactions in food can be chemical (oxidation or non-enzymatic browning reactions), microbial, biochemical (enzymatic browning, lipolysis, proteolysis...) and physical. They are influenced by two types of factors: the nature of the food and its surrounding. Understanding the reactions and knowing about the factors which control them are the first steps in developing food packaging which will minimise undesirable changes in quality and therefore protect the product (Roberston, 2010). This knowledge enables the packaging developers to choose the most suitable material resenting the required barrier properties, and thus ensuring a long shelf-life to the product (Marsh & Bugusu, 2008). The interactions between the product and the packaging also include potential migration of components from the packaging to the product, resulting from the movement of volatile or non-volatile substances from the packaging material into the food. They can be detected by taste or smell, or using analytical techniques (Corner & Paine, 2002).

Graph 2 shows the Oxygen Transmission Rate (OTR) of fifteen common polymers (Soarnol, s.d.). The OTR is defined as the volume of oxygen passing by a defined surface for one day. Therefore, the OTR is calculated for a given packaging. It is related to the material permeability via its thickness and the variation of pressure between the inside and the outside of the packaging (ΔP):

$$P = \frac{\text{OTR}}{\Delta p} \times \text{thickness}$$

Graph 2: OTR of different polymers



In this thesis, HDPE, polypropylene (PP) + ethylene vinyl alcohol (EVOH) and PP + EVOH + UV block were the four polymers or combinations of polymers studied. HDPE has the lowest permeability to oxygen, so it can be used for products which are resistant to oxygen and need to release gas during their storage, whereas PET and PP + EVOH, which are much more barrier should be used for sensitive products and products which do not produce too much CO_2 that would need to escape from the packaging.

Moreover, polymers do not absorb the same wavelengths. Clear HDPE and PE do not absorb UV, in opposite to glass: this makes the products vulnerable to UV when packed in these first polymers. Photo-sensitizers do not absorb all in the same area of UV and visible light. Therefore, it is important to know well the product and its components to be able to choose the most suitable packaging which will absorb the right wavelengths which are harmful to the product. Some anti-UV can also be added and change light transmission properties, as shown on Graph 3 (Duncan, van Aardt, & Marcy, 2001).

Graph 3: Light transmission spectra of four polymers combinations

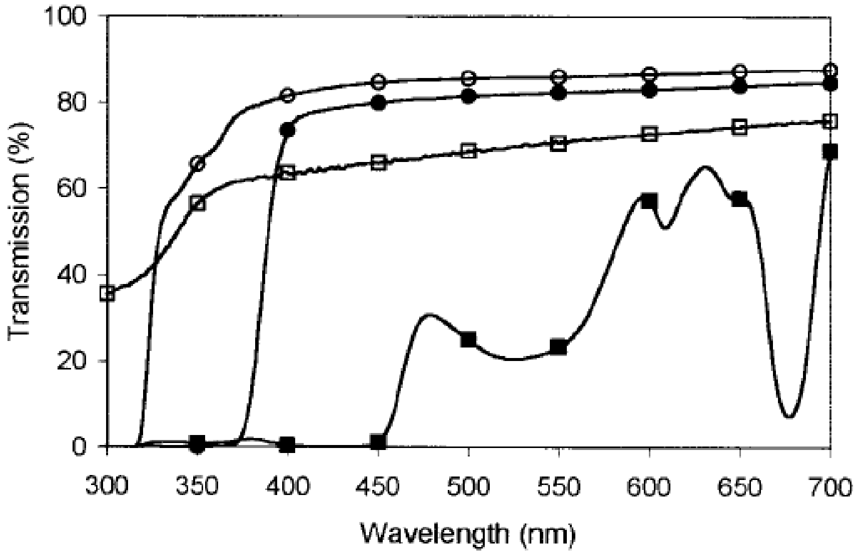


Figure 1. Light transmission spectra of high-density polyethylene (□), amber poly(ethylene terephthalate) (■), clear poly(ethylene terephthalate) (○), and clear poly(ethylene terephthalate) with UV light block (●).

These properties, gas permeability and light transmittance, influence the way they protect the product from the environment. If they can pass through packaging and if the product contains photo-sensitive molecules, it can provoke photo-oxidation of the product which may change its organoleptic, nutritional or visual properties.

3 Methods and measurements

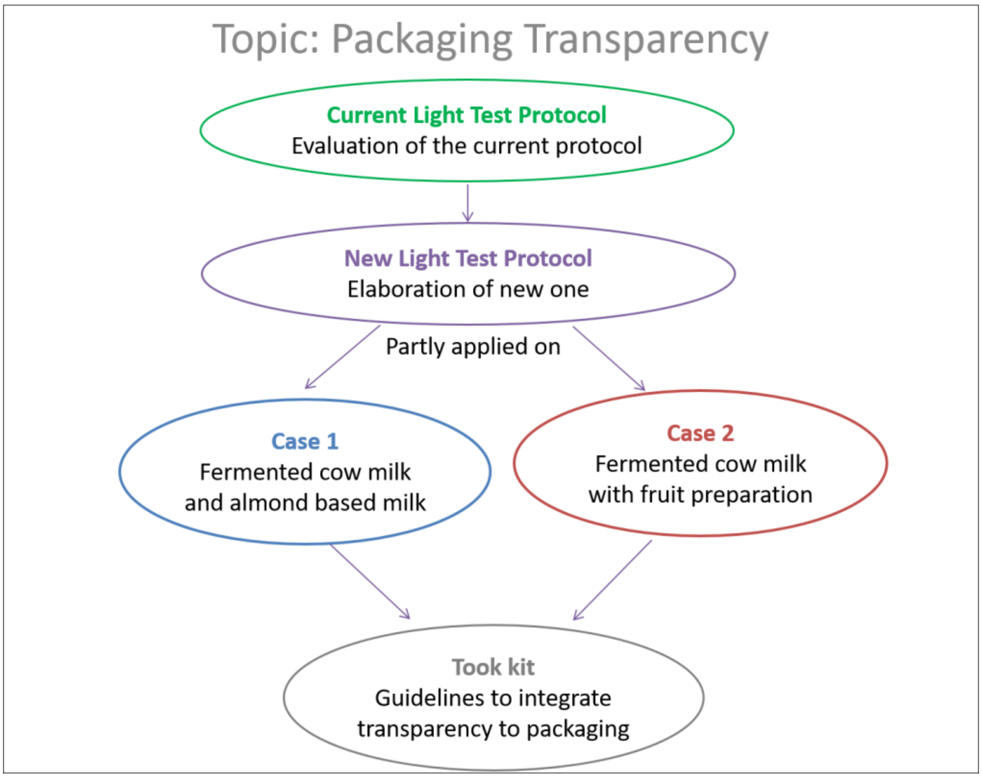
3.1 Overall research procedure

To understand the impact of light exposure on fermented products, an evaluation of the current light test protocol in Danone was first done, and a comparison with what was occurring in real supermarkets and in other Danone centres was established. Based on these results, a new light test protocol was defined. Part of this protocol was applied to two cases presenting four products and different packaging barriers.

- Case 1 studies two types of fermented milks: one fermented cow milk and one fermented almond-based milk.
- Case 2 is giving input on a fermented cow milk mixed with fruit and vegetable preparation. One red preparation and one green preparation were studied.

Both sensory and analytical monitoring were set up to follow the impact of light and oxygen on these products. Based on these results, the purpose of both cases was to find the barrier properties needed to integrate transparency into their packaging. The overall research procedure is summed up in Figure 3.

Figure 3: Overall research procedure



Depending on the case, packaging with no barrier, oxygen barrier and UV barrier were tested and six parameters were followed: organoleptic evaluation, gas chromatographic profile, pH, Dornic degree, colour and the headspace gas composition.

3.2 Light exposure test protocol

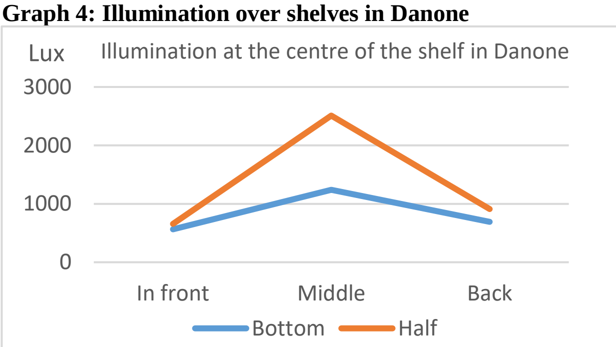
3.2.1 **Current light test protocol**

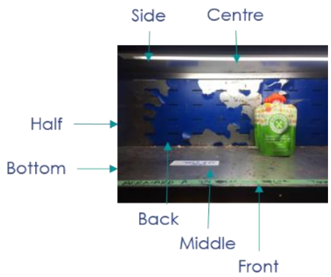
The current light test protocol in the company recommends exposing the products under fluorescent neon tubes (35W, 1500-2000 lux) over 5 days, with an alternating of 14 hours of light and 10 hours of darkness to mimic supermarket's opening hours. The same number of products is kept in the dark as reference, at 4°C.

After, five days, all products were gathered in a cold room, at 4°C, where they were kept until the end of the test, i.e. the end of shelf-life of the products with a margin of 30% (30 days + 30%, i.e. 40 days).

The characteristics of the light-exposed shelves, in Graph 4, where the tests took place were measured: illumination and temperature were the two parameters studied.

Graph 4: Illumination over shelves in Danone

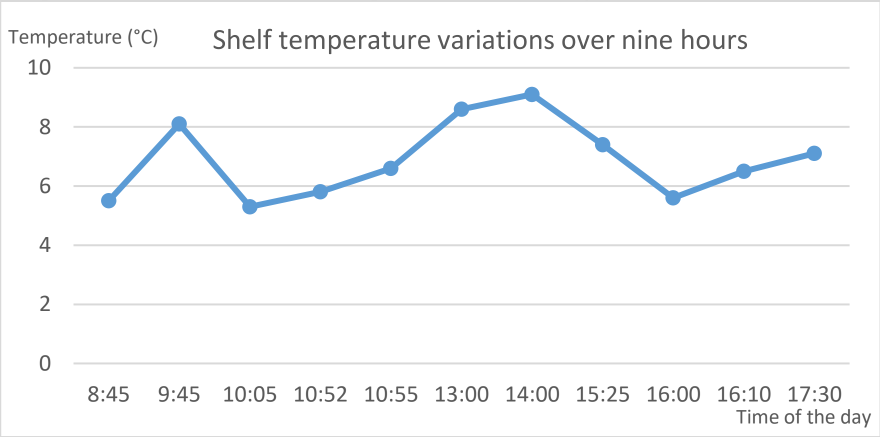




Illumination was measured at various places within the shelves. First, illumination depends on the distance from the source. This is why measures were done at the bottom of the shelf, meaning that the equipment was placed on the shelve, and at half the distance between the lamp and the bottom, to measure the illumination received by the product at this distance, which corresponds to the top of the bottles which were used in this study. Therefore, the total illumination received by the product was between these two measures: one bottle placed in the middle receives between 1200 and 2500 lux from its bottom to its top. During a light test, products are placed all over the shelf, so some of them are at the back of the shelf, whereas others are in the middle and last ones in the front. It is thus important to evaluate the variation of illumination over the shelf to know the variation in illumination received by all the products. One can see on Graph 4 that products placed in the back and in front receive the same amount of light. However, products placed in the middle, right below the tube, are more illuminated, going up to 2500 lux when the measure is taken at the half of the shelf.

Average temperature in the shelf was also measured over nine hours. Graph 5 shows that temperature is not constant during the day, and fluctuates between 5,3°C and 9,1°C.

Graph 5: Variations of the shelf temperature over one day



Considering all the variations on the current shelf, measurements were done in supermarkets to define better parameters.

3.2.2 **Improvement of the protocol**

An improved protocol has been developed based on real life measurements, calls with suppliers and a company internal benchmark. Inspiration was also taken from the literature review, in Chapter 1.

Measurements in supermarkets were done to check if the protocol was closed to the real conditions: type of lighting (LED or fluorescent, vertical and/or horizontal, correlated colour temperature (in Kelvin) …) and temperature in refrigerated display shelves were collected. Moreover, illumination (in lux) was measured with a LUX-meter. Phone calls with lighting suppliers were also conducted, to know what was the trend in term of lighting in supermarkets and to confirm what was observed during visits in supermarkets. Finally, internal benchmark was done within the company, to collect data about all the light test protocols used in other centres, departments or countries.

Due to a lack of time, the new light test was only partly applied on the two cases: the same shelf as described in 3.2.1 was used, with the same parameters (temperature, illumination, exposure duration) but the sensory test was coupled with analytical measurements.

3.3 Case 1 - Fermented cow and plant-based milks

3.3.1 **Case description**

The first case focuses on two drinkable yoghurts: one using cow milk fermented with classic yoghurt ferments, *Streptococcus Thermophilus* and *Lactococcus Bacillus*, and one using almond paste and only one vegan ferment. This last recipe was also supplemented with pea proteins and calcium, to have similar nutritional properties as dairy products, see Table 2.

Table 2: Products of Case 1

Type of product	***Dairy Product***	***Plant-based product***
Composition	Cow milk + ferments	Almond/ milk + vegan ferments + pea proteins + calcium (from seaweeds)

Table 3 summarizes the packaging options which were tested. Case 1 products were tested in PP bottles, with different added components to provide UV barrier, O_2 barrier or both. In a second phase, it was decided to fill the headspace with nitrogen to reduce the impact of oxygen. However, two trials showed that the equipment available made it impossible to decrease the oxygen content under 10%. Filling the bottles at the maximum was found to be the closest accessible solution. Therefore, these products were packed with very little headspace. According to a study made by Mortensen et al, samples stored in nitrogen will have less photo-oxidation, since type II reactions are impossible. However, type I reactions still occur at very low oxygen concentration (0,2%) and can provoke off-notes in the light-exposed products (Mortensen, Sorensen, & Stapelfeldt, 2003).

Table 3: Experimentation plan for Case 1

	In the dark (reference)	PP + EVOH	PP + EVOH + 1% Anti UV	PP + EVOH + no headspace	PP + EVOH + 1% Anti UV + no headspace
In the dark (reference)					

PP + EVOH	Impact visible light + UV				
PP + EVOH + 1% Anti UV	Impact visible light only	Impact of UV only			
PP + EVOH + no headspace	See if there is protection when no oxygen at all but UV	Impact O_2 in the headspace			
PP + EVOH + 1% Anti-UV + no headspace	See if there is protection when no oxygen at all and no UV		Impact O_2 in the headspace when no UV		

For all these options, an organoleptic evaluation was conducted alongside with gas chromatography, to correlate off-notes to photo-oxidation mechanisms. pH, Dornic degree, colour and headspace gas composition were also measured. Every test was led just after the light exposure test, which corresponds approximatively to the middle of the shelf life (12 days), the end of the shelf life (30 days) and 10 days after the end of the shelf-life (40 days).

3.3.2 **Organoleptic evaluation**

The main objective of the organoleptic evaluation was to detect oxidised aroma, rancidity or acidity which would help understand if oxygen and light influenced the product. A questionnaire was defined beforehand. A panel of 6 people was chosen for the whole test and each of them had to try both samples, exposed and not exposed to the light, without knowing which one they were tasting. To remove the bias coming from the degustation order, participants were told to follow the degustation order written on their questionnaires (with an anonymous code on the samples). All participants had a different order. The questionnaire used is in Appendix A.

3.3.3 **Gas chromatographic profile**

An aromatic profile was done by gas chromatography and had the following objective: detect the volatile compounds to link them with the organoleptic evaluation and potentially detect compounds that are not perceived by the sensory panel but might present a risk to develop in the product later on or if stored in extreme conditions by the consumer. This was carefully compared to the sensory test, to link the potential perceived off-notes with molecules, and therefore understand the mechanism which caused the apparition of the off-note.

3.3.4 **pH measurement**

pH evolution over shelf-life usually comes from the ferments' activity. According to the interview led with experts in ferments and the different experiments which have been done within Danone, ferments do not seem to react with light. When it comes to storage, the instructions usually tell to keep them in a dry, dark and cold space. However, inside the product, the matrix usually opaque blocks a large part of the light which does not affect the ferments. What may raise problems though, is the permeability of packaging to gas. If the packaging is too much barrier, O_2 cannot enter the packaging while it is necessary to transform some precursors produced by the ferments into volatile molecules, such as diacetyl which is responsible for creamy and butter flavour in yoghurts. But ferments are mostly anaerobic. Thus, a total absence of O_2 could lead to an activity of the ferments during storage which would lead to a post-acidification of the product. On the contrary, if the packaging is not barrier at all, too much O_2 can enter and threaten the ferments' survival This is why pH was important to follow alongside with sensory and gas chromatography tests, to check ferment's activity and survival regarding packaging permeability. A pH-meter TOLEDO SevenEasy^{TM} was used for the measures. Duplicates were done for each measure.

3.3.5 **Dornic degree**

In this case, lactic acid is the main acid present in each product, as a fermentation product. Dornic degree was thus a good parameter to evaluate ferments' activity in the dark and exposed to light and completes the result found with pH measure: indeed, it gives the amount of only one acidic molecule, partly responsible for pH decrease in fermented products: the lactic acid. Thus, it gives a relevant information of ferments' activity. 1°D is equivalent to 0,1g/L of lactic acid. A Metrohm 855 Robotic Titrosampler was used for these measures. Duplicates were done for each measure.

3.3.6 **Colour measurement**

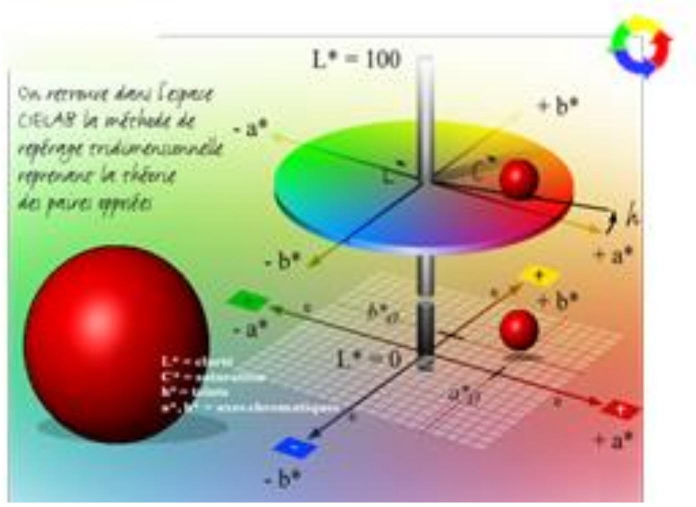
Fermented cow milk and almond-based milk are respectively white and beige. The follow-up of colour was mainly to follow potential maillardisation, caused by the reaction of proteins with sugars which would provoke browning of the product.

The colour was measured on the same sample as the one used for pH-metre. A portable spectro-colorimeter Data Check was used, and gave three data from the Cielab space (see Figure 4) to describe a colour: h° (hue angle), L* (clarity) and C* (chroma). It also provides DE between two colours: this value enables to tell if a naked eye can differentiate two colours. If DE<1, it is hardly impossible to distinguish them; if 1<DE<5, it is possible to distinguish the two colours and the difference between them is proportional to DE, and if DE>5, the colours are different and no more proportional comparison is possible. This measure helped thus, define if light and oxygen had an impact on the product's colour evolution and if this difference was perceptible. Depending on the method and the products, different DE are calculated. Here is the formula for the DE used for white products:

$$DE_{CIE2000} = [(\Delta L^*/k_L S_L)^2 + (\Delta C^*/k_C S_C)^2 + (\Delta h°/S_H)^2 + R_T(\Delta C^* \Delta h°)/(S_C S_H))]^{1/2}$$

k_L, k_C and k_H 3 coefficients depending on clarity, chroma and hue, enabling an optimal adjustment regarding the product; in this method, k_L, k_C, k_H are equal to 1. S is the surface of product on which the measure is done. R_T is the rotational term, depending on C* and hue angle.

Figure 4: CIELAB Space



For each sample, twelve measures were done: three tanks per reference, and four measures per tank. Uncertainty measures were calculated for each product, to know the variability between samples and the precision of the measure, which depends on the product's matrix, the operator and the equipment.

3.3.7 **Gas composition in the headspace - O_2 & CO_2**

O_2 measurement in products packed with a headspace was a good indicator of product's oxidation, making the hypothesis that oxidation of the product would mainly come from oxygen dissolved in the product, which was difficult to measure, and from oxygen in the headspace, since all the bottles used in this case were barrier to gas. CO_2 measurement was only an indication of contamination of the products, but did not give any information about ferments activity during storage since *Streptococcus Thermophilus* and *Lactococcus bacillus* are homofermentative and thus do not produce CO_2. O_2 and CO_2 contents were measured in the headspace in the bottle used for organoleptic evaluation. An ABYSS® gas analyser was used.

3.4 Case 2 – Fermented cow milk with fruit and vegetable preparation

3.4.1 **Case description**

The second case regards fermented cow milk, mixed with fruit and vegetable puree. 60% of fermented milk was mixed either with 40% of red preparation or 40% of green preparation. Both preparations contained a mix of at least five different fruits and vegetables. No sugar, aroma or stabilizer were added to the final recipe. A specific ferment, which will be called thereafter ferment X, was added besides the classic yogurt ferments, *Streptococcus Thermophilus* and *Lactococcus Bacillus*. Once again, a transparent packaging would enable the consumer to see the fruits inside. This case provided new insights since fruits and vegetables contain different vitamins, acids and pigments compared to dairy products, which can react with light, or might on the contrary decrease the impact of light on the white mass. Furthermore, a new ferment was used here, with some particularities. For example, this ferment can ferment malic acid from the fruit preparation: this fermentation is closed to yeast fermentation and can generate CO_2. This adds a constraint on the packaging barriers needed compared to Case 1.

All packaging options are summarized in Table 4. For this case, two materials were used: HDPE + 1% anti-UV, which is a poor barrier to oxygen, and PP + EVOH + 1% anti-UV, which is a very good barrier to oxygen. Both were stored under light and in the dark, as references.

Table 4: Experimentation plan for Case 2

	PP + EVOH + 1% Anti UV in the dark	HDPE + 1% Anti UV in the dark	PP + EVOH + 1% Anti UV Light exposed	HDPE + 1% Anti-UV Light exposed
PP + EVOH + 1% Anti UV In the dark				
HDPE + 1% Anti UV In the dark				
PP + EVOH + 1% Anti UV Light exposed	Impact visible light only			
HDPE + 1% Anti-UV Light exposed	Impact oxygen only when no light	Impact light and oxygen	Impact oxygen only when light	

For all these options, an organoleptic evaluation was conducted as well as pH, colour and headspace gas composition measurements. Every test was led just after the light exposure test, which corresponds approximatively to the middle of the shelf life (12 days), the end of the shelf life (30 days) and 10 days after the end of the shelf-life (40 days).

3.4.2 **Organoleptic evaluation**

In that second case, an organoleptic evaluation was led, but was not correlated with a gas chromatography. The recipes were too far from the final ones: undertaking such an analysis was too early for Danone company at this stage. The method used was the same as the one used in Case 1. The questionnaire for the sensory test is in Appendix B.

3.4.3 **pH**

In this case, pH measurement was not only a tool to follow ferments' activity. If the material is too much barrier to gas, the risk is that CO_2, which can be produced by ferment X, cannot escape from the bottle and is dissolved into the white mass, which provokes post-acidification. The CO_2 produced can also lead to a "pop" sound when the consumer opens the lid, impacting the SMOT. Furthermore, the lid takes a dome shape, which may not be expected. Both post-acidification and pop sound can affect the consumer experience and therefore need to be controlled. Following the pH was a good indication of the production of CO_2 since its dissolution inside the product provokes a decrease in pH. A pH-meter TOLEDO SevenEasy™ was used for the measures. Dornic degree was not relevant in that case since lactic acid was not majoritarian. The result would have therefore had no meaning.

3.4.4 **Colour**

In this case, products were red and green. Therefore, colour was a very important parameter to evaluate in this case, compared to Case 1. Indeed, in transparent bottles, the colour of the product is one of the first property seen by the consumer during the FMOT. If colour is unstable during storage, it can disappoint the consumer and have a negative impact from a SMOT perspective: in that case, the consumer may not repurchase the product. On the other hand, if differences in colour are noticed during the exposition in supermarket refrigerated display, it would trigger variability in colour between the products on the shelf if some have been exposed longer than others. This would influence consumer's intention of buying from a FMOT perspective instead. For this measure, the same equipment as for Case 1 was used. L*, C* and h° were also measured. However, another DE, specific to non-white products, was calculated:

$$DE_{CMC} = [(\Delta L^*/k_L S_L)^2 + (\Delta C^*/k_C S_C)^2 + (\Delta h°/S_H)^2]^{1/2}$$

k_L and k_C, 2 coefficients depending on clarity and chroma, enabling an optimal adjustment regarding the product, and S the surface of product on which the measure is done. In this method, k_L and k_C are equal to 1.

3.4.5 **Gas composition in the headspace - O_2 & CO_2**

As mentioned earlier, CO_2 is one product of the fermentation in this case. Therefore, unlike case 1, measuring this gas in the headspace was a good indicator of the ferments activity. O_2 content was also measured to see if product consumes oxygen in the headspace during storage, and to potentially correlate it with oxidised off-notes. O_2 and CO_2 contents were measured in the headspace in the bottle used for organoleptic evaluation. An ABYSS® gas analyser was used.

4 Results and discussion

4.1 Light Test Protocol

4.1.1 **Conditions in supermarkets**

Different French supermarkets (Carrefour City, Monoprix, Leader Price and Naturalia) were visited and illumination measurements were done. One first remark was that many supermarkets are turning to LED, rather than fluorescent light which consume more energy. This was confirmed by a world-wide light supplier, which sells hardly only LED lights to supermarkets nowadays and plan to sell 100% LED light by 2022.

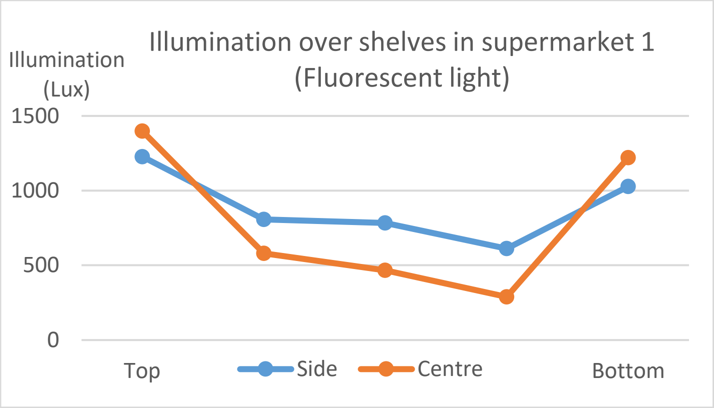
Still to save energy, a lot of supermarkets have closed fridge, see Picture 2. This has two main effects; on one hand UV light from the general supermarket light cannot penetrate through the glass of the fridge and therefore cannot reach and damage the products. On the other hand, it enables to put vertical tubes, having a homogenous lighting compared to horizontal tubes which are sometimes only on the top of the fridge.

Picture 2: Close fridges with vertical LED tubes (on the right) and open fridge with horizontal fluorescent tubes (on the left) in two different supermarkets

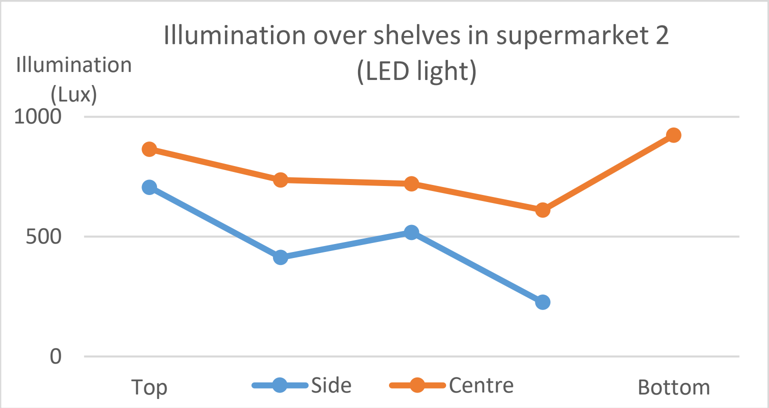
Besides the type of light, its characteristics were noted: the illumination (in lux) and the correlated colour temperature (in Kelvin). Most of refrigerated shelves in supermarkets have an illumination between 1000 and 1500 lux (see Graph 6 and

Graph 7). Illumination is generally lower when LED technology is used. This was confirmed by a study made by Smart Light Engineering, recommending for cabinet displays in retails and supermarkets an illumination of 1000 lux (Smart Light Engineering).

Graph 6: Illumination over five shelves in supermarket 1, illuminated with fluorescent light



Graph 7: Illumination over five shelves in supermarket 2, illuminated with LED light



For dairy products, like yoghurts, it was observed that supermarkets usually use cool white light (CCT: 4000K) or more occasionally daylight (CCT: 6500K). Cool white, in opposition to warm light (CCT: 2700K), is well-adapted to white fresh products, since these products will look fresher under cool white light.

Based on this information, LED and fluorescent lights, with a Correlated Colour Temperature of 4000K and an illumination between 500 and 1500 lux are the recommendations which were done as an equipment to lead light tests on dairy products.

4.1.2 **Internal benchmark**

Finally, discussions were led with different teams in Danone Research, to compare the methods used. It was found that all European countries use the same protocol, which is the one used in Palaiseau. One difference though was observed in Poland, where storage temperature is 10°C instead of 6°C in the other countries. This may be due to specificities in Polish supermarkets or fridges.

It was also decided to challenge the five days of light exposure suggested in the protocol, to know if it was based on strong marketing studies or just an indication. Therefore, the logistics team of Palaiseau France was contacted to have an estimation of the turn-over of Danone's products in French supermarkets. A study was done over 214 Carrefour hypermarkets, 120 Auchan hypermarkets and 200 Intermarché supermarkets. They found that on average, Danone products stay 4 days in Carrefour hypermarkets, 4,5 days in Auchan hypermarkets and 5,5 days in Intermarché supermarkets. Therefore, suggesting 5 days in the protocol seems relevant. Nevertheless, this duration is to be re-evaluated within each project since the previous study was done mostly on big supermarkets, which have a bigger turn-over compared to city-centre markets. Depending on the product, on the target and on the location, this duration may be modified.

4.1.3 **New Light Test Protocol**

Based on the previous results and on the current protocol described in 3.2.1., a new protocol was established to evaluate the impact of light and oxygen on food products stored in cabinet displays in supermarkets.

First of all, considering the increase of LED tubes in cabinet displays and their characteristics compared with fluorescent light, the two types of lights are now proposed in the protocol. This enables to have conditions closer to the real life, but also to compare the impact of two types of lighting on the product's evolution, mostly since LED are not emitting in UV and produce less heat than fluorescent light.

Secondly, two purposes were defined for the light test protocol, according to the stage of the project: the first one is to "UNDERSTAND" the impact of light and oxygen on the product, in the early stages of the packaging and product development, to define if transparency is accessible regarding the product's sensitivity, and to evaluate the level of accessibility. Therefore, during the stage "UNDERSTAND", products will be placed 5 cm one from each other, so they all receive the same amount of light and it will thus be possible to compare the measurements which are done on each bottle. The other purpose it to "VALIDATE" the final packaging, when it is transparent, in later stages of development such as implementation. During this test, products will be grouped together, in the same way as they are in supermarkets, with a pick-up if there is one. The aim of this stage is to check that products do not develop off-notes and are not damaged in terms of nutritional value, colour… during storage in supermarket, i.e. within their final packaging, in the same disposition as in retail outlets. Moreover, during the stage “UNDERSTAND”, both LED and fluorescent lights can be tested, to understand UV impact for instance, whereas during the stage “VALIDATE”, only the light under which the product will be exposed in real life, depending on the country and type of supermarket, will be tested. Table 5 sums up the particularities for each phase.

Table 5: Light Test Particularities according to the project phase

Stage	Stage “UNDERSTAND” *Phases DEFINE & DEVELOP*	Stage “VALIDATE” *Phases IMPLEMENT, LAUNCH & POST-LAUNCH*
Purpose	Understand the impact of light, UV and oxygen on the stability of under development product when stored in transparent packaging, to give design guidelines to the packaging and product developers. The evaluation helps to determine the accessibility of the transparency for the developed product. Such evaluation is mandatory to define packaging specifications.	Check the product stability with the full pack system (primary, secondary and tertiary pack when relevant (Shelf Ready Trays)) when exposed to shelf light and until the end of the shelf life.
Positioning	Bottles need to have all the same light exposure. Samples will be placed 5 cm from each other to be fully lighted.	The purpose here is to check the real conditions The samples will be grouped together, in the same way as they are in supermarkets, with a pick-up if there is one.
Type of lighting	LED **and** Fluorescent light tests will be led in parallel.	LED **or** Fluorescent light test will be led depending on the context.

Finally, for both stages, analytical tests coupled with an organoleptic evaluation will be done. Gas chromatography, pH and Dornic degree, gas analysis in the headspace and colour are the ones recommended. Others, such as bacteria counting, internal pressure, follow-up of vitamins, etc can be added depending on the product. In the same way, some of the measurements listed above can be removed if no relevant. In this thesis, protocol "UNDERSTAND" was partly applied to cases 1 and 2. Both analytical and sensory tests were done, and products were placed in their primary packaging 5 cm each one from each other. However, LED lamps were not available. Therefore, only fluorescent tubes were tested.

4.2 Case 1 – Fermented cow and plant-based milks

4.2.1 **Organoleptic evaluation**

4.2.1.1 With headspace

<u>Cow milk:</u>

Dairy products exposed to light did not present off-taste at the middle of the shelf-life, although the one with a protection against UV light and oxygen was rated as flatter, meaning with less strong taste than the others. Moreover, the reference stored in the dark had a citrus taste that the others did not have. At the end of the shelf-life, the differences between the samples had increased. Terms such as "old cheese", "rancid" or "plastic" were mentioned by some participants. Finally, more off-notes were found in the sample exposed to the light with a protection against O_2 and UV than the one with a protection against O_2 only. One hypothesis to explain this observation is that the UV block may reduce the efficiency of the oxygen barrier. Another hypothesis would be that the anti-UV additive which is added into the bottle is migrating into the product, the migration being enhanced by light, and gives off-notes to the product. In any case, visible light seems to have more impact on the off-notes development than UV wavelengths.

<u>Almond-based product:</u>

Regarding almond-based products, differences between samples exposed to light and the ones stored in the dark started to appear earlier, from the middle of the shelf-life. Cardboard, metallic off-notes were mentioned for the ones exposed to light. The reference one was defined as sweeter and with a citrus taste, like in dairy products. Moreover, this time again the UV block did not seem to improve the organoleptic properties of the product since off-notes were detected in both samples exposed to the light. These observations were confirmed at the end of the shelf-life.

Since cardboard and metallic off-notes are typical of oxidation reactions, a second test was led with products with a very small headspace, to decrease the impact of oxygen and confirm or infirm the previous results.

4.2.1.2 Without headspace

Overall, removing the headspace, thus an important source of oxygen in contact with the product, improved the organoleptic properties of the ferment cow and almond milks exposed to the light. Nevertheless, some differences persist, which may be explained by type I photo-oxidation reactions, occurring at lower oxygen concentration, or by the oxygen dissolved in the product. This dissolved oxygen may be higher in the tests led during this thesis than in industrial conditions, since products were done at a pilot scale, with manual filling.

<u>Cow milk</u>:

At day 13, every participant rated the reference stored in the dark as the best one. Rancidity, old cheese and farm notes were detected in the one with only O_2 protection. This tendency was confirmed at day 30 and day 42, with a citrus taste at the end for the reference. But the differences were less perceptible than between the samples with a headspace. The fact that off-notes were perceived after 13 days already whereas no off-notes was detected during the test with headspace may be explained by the panel experience: with time, it became easier for them to detect oxidation off-notes.

<u>Almond-based product</u>:

At day 13, just after the five days of exposure to the light, all participants could detect differences between the sample stored in the dark and the ones exposed to light. Metallic and rancid off-notes were detected. The sample without UV protection was found as waterier than the others, whereas the one with the protection UV, once again, was rated as more rancid and metallic. On the contrary, the reference had a strong almond taste, even stronger than the reference at day 12 with a headspace. The absence of oxygen seems to protect almond aroma. However, at day 30 and 42, the differences between the three samples were less important. Beany, cardboard and butter notes were detected at day 30 in the samples exposed to light, but without a real consensus within the participants, and it became quite difficult to distinguish the three samples at day 42. It seems that light has a strong impact on the organoleptic properties of the product during the exposure, so when tasted right after they are removed from the shelf line-up, the differences are quite important. However, in absence of headspace, the product seems to evolve in quite a comparable way, exposed or not to the light before. Moreover, the metallic taste can also come from the pea proteins, rather than the lipid oxidation, since source of oxygen was reduced: the only source of oxygen was the oxygen dissolved in the product, which may come from the production and conditioning steps, which were done manually. The gas chromatography was used to give insight on this off-taste.

4.2.2 **Gas Chromatography**

Gas chromatography gives access to absorption peaks which can be linked with volatiles compounds. The size of these peaks enables to compare the amount of one molecule in several products. However, one needs to keep in mind that it is not possible to compare molecules among themselves. In one product, if the absorption peak of one molecule is twice higher than the absorption peak of another, it does not mean that this molecule is present in the product in twice a higher amount than the other. No comparison is possible. However, if the absorption peak of one molecule is twice higher in a product than in another, this does mean than the first product contains twice more of this molecule than the second product.

All the compounds observed below were present in tiny amounts. However, their detection threshold is very low. Therefore, they were well correlated with sensory tests.

In cow and almond milks exposed to light, the important volatile compounds measured by gas chromatography are 2-heptanone, hexanal, heptenal, 2-butanal, 2-nonenal, pentanal, propanal, 1-octen-3-ol and dimethyl disulfide (Intawiwat N. , 2011).

4.2.2.1 With headspace

<u>Cow milk</u>

A much higher amount of dimethyl disulfide were detected in the dairy products exposed to the light, mostly at the end of the shelf-life, compared to the reference stored in the dark. Aldehydes were also observed in all samples at the beginning and disappeared in the reference after 30 days, whereas they were still present in the ones exposed to the dark, mainly unsaturated aldehydes. All these compounds explain the rancidity and "old cheese" notes observed in the samples exposed to the light during the sensory test. Furthermore, dimethyl disulfide is a photo-oxidation marker, and confirms that photo-oxidation occurred in samples exposed to light, both with or without UV barrier.

On the other hand, the citrus taste observed in the reference is explained by the presence of acetaldehyde, acetone and 2-butanone which bring freshness to the product. These molecules were observed in the samples exposed to light as well, but may be hidden during the sensory test by the other compounds described above.

<u>Almond-base product:</u>

Regarding almond-based products, the gas chromatography showed at the end of the shelf-life in the reference stored in the dark a higher quantity of benzaldehyde and a smaller number of aldehydes which give green, rancid and bean off-notes compared to light exposed products.

Moreover, a high concentration in 1-octen-3-one was observed in the samples exposed to the light and explains the metallic note. This molecule is a degradation product of lipids oxidation (linoleic acid), either by enzymatic means or by oxygen. Both observations confirm that lipid oxidation occurred in samples exposed to light, with or without UV barrier.

One other general conclusion from both organoleptic evaluation and chromatography gas is that UV block does not help protect the product, and in some way, seems to damage even more the product than without this barrier. Visible light seems thus to have a considerable impact on the product, while UV light does not. What remains difficult to explicate though, is the fact that both cow milk and almond milk have more off-notes when exposed to light when they have the UV barrier, whereas one could expect that if UV does not have any impact, both products exposed to light with and without UV block would have the same evolution. Two hypotheses can be formulated at this stage regarding these results: either the UV block combined with the oxygen barrier decrease the efficiency of the oxygen barrier, or, given the illumination and temperature variability over the shelf, products with UV barrier were stored during the five days on the shelf in worst conditions than the products packed without UV barrier (see Graph 4 and Graph 5).

4.2.2.2 Without headspace

Gas chromatography on samples without headspace showed an evident link between the volatile organic compounds and the sensory test, like on samples with a headspace. Globally, the same evolution over time as the evolution for samples with a headspace is observed. However, the differences between samples exposed to the light and samples stored in the dark are less important than the differences observed in the first test. This test confirmed the hypothesis made during the first one with headspace, that UV barrier does not protect the product against photo-oxidation.

<u>Cow milk</u>:

Regarding sulfuric compounds, there is a higher quantity of dimethyl disulfide in the samples exposed to the light, but the difference between the reference stored in the dark and the light exposed ones tends to soften with time. This compound is a photo oxidation marker which has a very low threshold and can be correlated to the "old cheese" and "cow" off-notes described during the sensory test. However, the differences between the reference and the samples exposed to the light are lower than the ones between samples packed with a headspace. Removing the headspace seems thus here to have an impact on product's oxidation.

A higher quantity of aldehydes is also observed in the samples exposed to the light: these compounds can be correlated to carboard and rancid off-notes, which were mentioned during the sensory test. Moreover, in the three samples their quantity increased with time.

However, on the contrary to samples with a headspace, no difference was observed regarding fermentation metabolites and methyl cetones. Removing the headspace seems thus to help preventing the dairy product from organoleptic changes.

Almond-based product:

Like for the almond products with headspace, there is a higher quantity of 1-Octen-3-one in the samples exposed to the light, bringing earthy and metallic notes. Moreover, its quantity is increasing over time in both samples exposed to light, with UV block and without it, reinforcing the hypothesis that most of photo-oxidation happens in the visible part of the light, and not in the UV.

On the overall, the gas chromatography confirmed what was observed during the sensory test: samples exposed to the light without headspace present photo-oxidation markers which bring off-notes such as rancidity and old cheese in dairy products and metallic notes in almond-based products. However, the differences are lower than the differences between products packed with headspace. Moreover, removing the headspace prevent fermentation metabolites in dairy products, softening the differences between the samples. Finally, this second test reinforces the hypothesis that visible light has an impact on photo-oxidation on these products, rather than UV wavelengths.

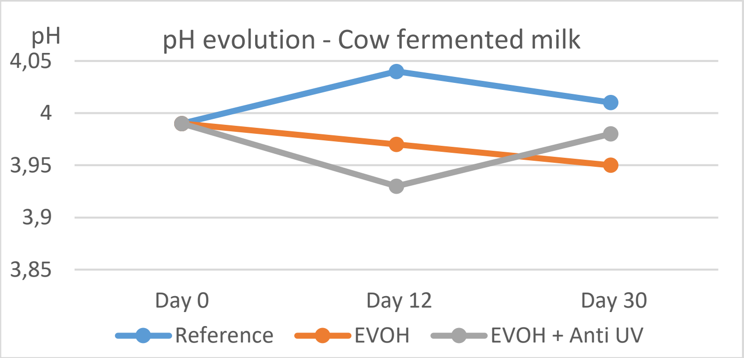
4.2.3 **pH**

4.2.3.1.1 With headspace

Cow milk:

In dairy milk, no clear evolution of the pH can be noticed in the reference stored in the dark and in the sample exposed to light with UV barrier, see Graph 8. pH evolution cannot explain the citrus taste which was felt during sensory test. However, a slight post-acidification was noticed over time for the fermented cow, which remains very low and not significative.

Graph 8: pH evolution for dairy products packed in PP + EVOH, PP + EVOH + anti UV, both exposed to light, and in the reference kept in the dark



Almond-based product:

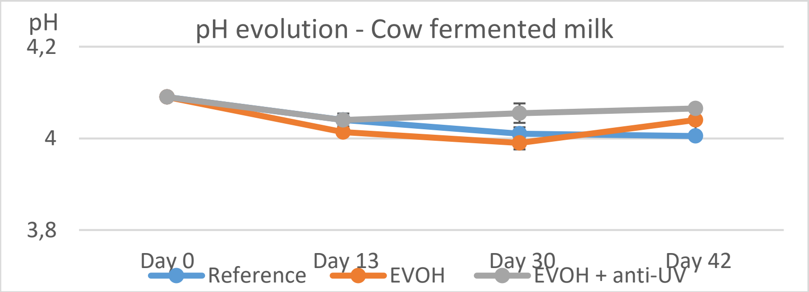
pH remains constant in almond-based product, which seems to indicate that ferments do not grow anymore during storage, when exposed to light, visible light only or even stored in the dark. This was confirmed in the following section on Dornic degree. Graphs are in Appendix C.

4.2.3.2 Without headspace

Cow milk:

Graph 9 shows that in dairy fermented milks, exposed to light or stored in the dark, pH remains between 3,99 and 4,09. There is thus no significant post-acidification in the product. The ferments being anaerobic, a post-acidification could have been expected without headspace, but it does not seem to impact the ferments activity.

Graph 9: pH evolution for milk products packed without headspace in PP + EVOH, PP + EVOH + anti UV, both exposed to light, and in the reference kept in the dark



Almond-based product:

pH remained constant over time, whatever the barrier in the pack, see graph in Appendix C. In accordance with what was found in products packed with headspace, ferments, even in full anaerobia do not grow on vegetal matrices. With or without headspace, with or without UV barrier and exposed under or in the dark, pH evolution does not differ.

4.2.4 **Dornic degree**

4.2.4.1 With headspace

Cow milk:

Table 6: Dornic degree evolution over time for dairy product

Cow milk	Day 12	Day 30
REFERENCE	57,1 °D	55,5 °D
EVOH	57,5 °D	60,5 °D
EVOH + Anti UV	52,0°D	51,9 °D

The sample packed in PP + EVOH + anti-UV has a much lower content of lactic acid that the two others. No explanation was found. This might come from the samples variability.

Almond-based product:

Table 7: Dornic degree evolution over time for almond based product

Almond based milk	Day 12	Day 30
REFERENCE	23,1 °D	23,5 °D
EVOH	22,3 °D	23,4 °D
EVOH + Anti UV	23,9 °D	23,5 °D

In almond-based products, concentration of lactic acid remains constant, over time and with no differences regarding the packaging barrier and the exposure to light. This confirms that fermentation does not occur anymore once the product is ageing: this is logical knowing that vegetal matrix does not provide any substrate for the remaining ferments.

4.2.4.2 Without headspace

Cow milk:

Table 8: Dornic degree evolution over time for dairy product packed without headspace

Cow milk	Day 13	Day 30	Day 42
REFERENCE	69,4 °D	70,1 ± 0,1°D	72,7 ± 1,8 °D
EVOH	71,9 °D	72,2 ± 1,0 °D	70,7 ± 1,0 °D
EVOH + Anti UV	69,1 °D	67,2 ± 1,6 °D	70,0 ± 0,0 °D

The Dornic degree in ferment cow milks is higher when there is no headspace than with headspace. This confirms the first hypothesis which was made regarding ferments activity: without any oxygen, their activity is more intense and they produce more lactic acid, even though no clear difference in pH was observed. However, there is no clear conclusion about the impact of light or UV on acidity evolution.

Almond-based product:

Table 9: Dornic degree evolution over time for almond based product packed without headspace

Almond-based milk	Day 13	Day 30	Day 42
REFERENCE	29,0 °D	28,8 ± 0,1°D	29,6 ± 0,2 °D
EVOH	28,1 °D	28,4 ± 0,3°D	32,1 ± 3,1 °D
EVOH + Anti UV	30,0 °D	29,0 ± 0,2 °D	29,7 ± 0,3 °D

In the same way as for almond products packed with headspace, Dornic degree does not evolve significantly. This confirms once again than vegetal ferments are inactive during storage and do not grow anymore.

4.2.5 **Colour**

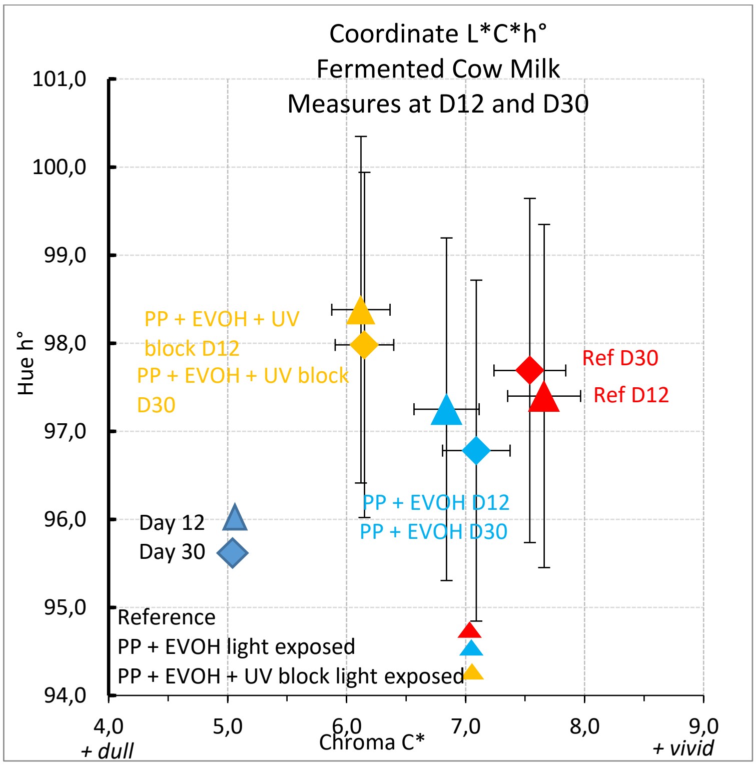
4.2.5.1 With headspace

Cow milk:

According to Graph 10, the hue of the product is not evolving over time and is not significantly different from one pack option to another.

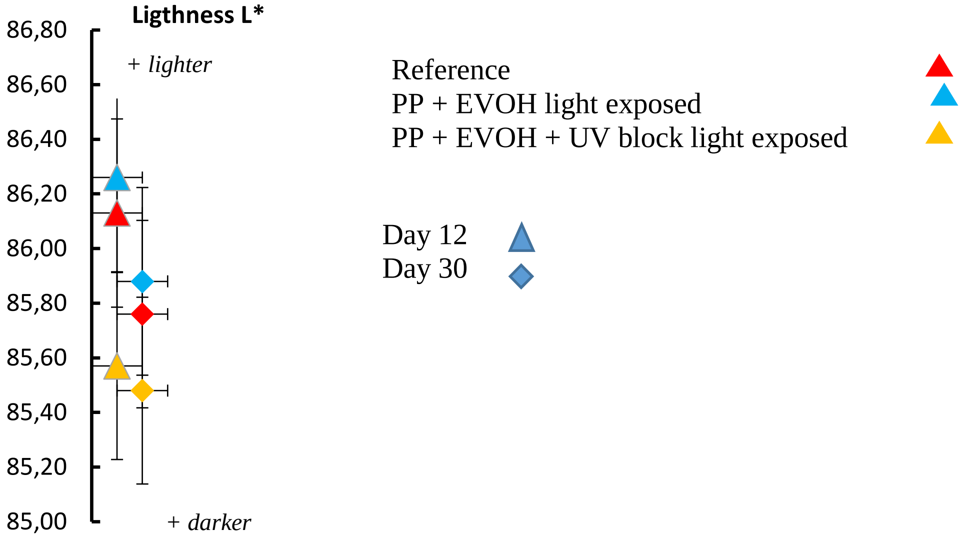
Regarding the chroma, significant differences can be noted : light seems to decrease the colour’s intensity of dairy fermented products. When exposed to light, fermented cow milk became duller. Moreover, UV block seems to enhance this effect. Visible light would therefore be more harmful for product's colour evolution than UV light, and UV light would even protect the product. There is no clear explanation to this. A last observation is that the loss of colour seems to happen during the first half of the product life : no more evolution of the chroma is measured between 12 and 30 days. Error bars were calculated based on the uncertainty measurements which were done beforehand and explained in Section 3.3.6.

Graph 10: C* and h° evolution over time at Day 12 and Day 30 and between reference stored in the dark, PP + EVOH exposed to light and PP + EVOH + UV block exposed to light, for dairy products



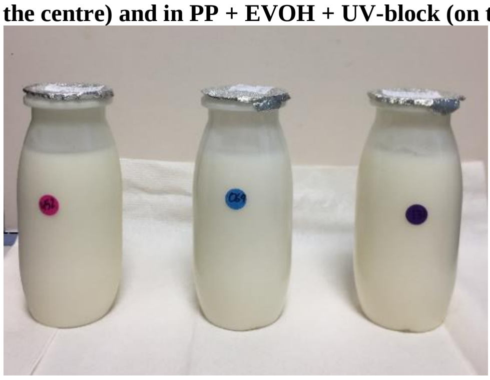
Differences in lightness however are not significative, see Graph 11.

Graph 11: L* evolution over time at Day 12 and Day 30 and between reference stored in the dark, PP + EVOH exposed to light and PP + EVOH + UV block exposed to light



To know if these differences can be seen to the naked eye, DE parameter needs to be analysed in parallel. If DE > 1, these differences are noticeable. DE between the samples over time stays very low, which means that the evolution of the product observed over time, seems not to be observable to the naked eye. However, DE between pack options is between 0,6 and 1, which means that only trained eyes would be able to make the differences, see Picture 3.

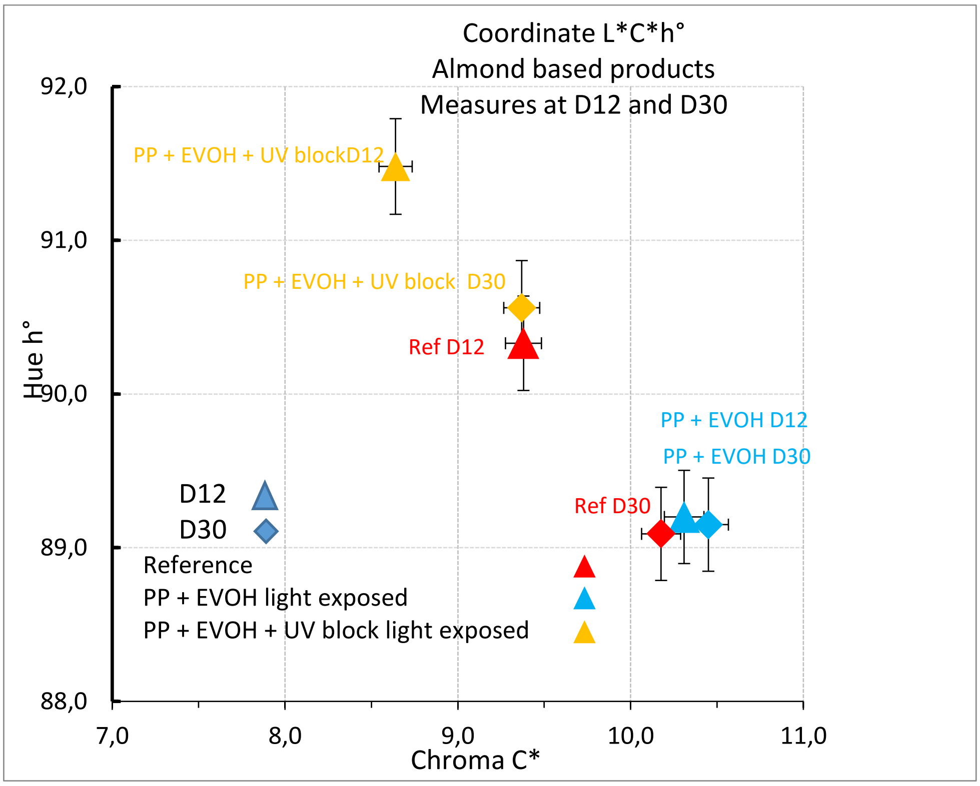
Picture 3: Fermented cow milk in packaging stored in the dark (on the left), in PP + EVOH (in the centre) and in PP + EVOH + UV-block (on the right), at D12



Almond-based product:

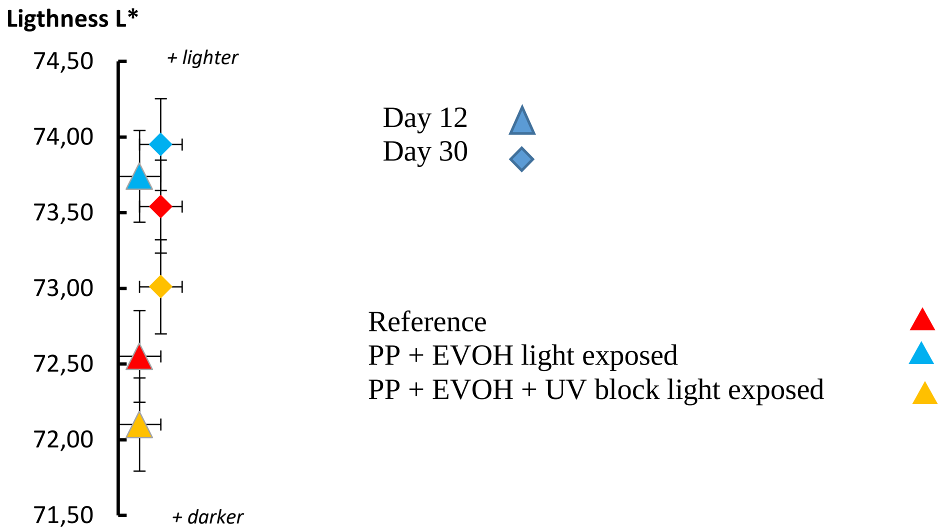
According to Graph 12, hue and chroma of the plant-based milk evolved over time and from packagaing options. First, like dairy proucts, chroma is increasing over time, and products become more vivid, mostly for products stored in the dark or exposed to the light without any protection against UV light. Hue, in opposition to dairy products, is evolving in a significant way when products do not have any protection against UV wavelenghts: these products have a lower hue, meaning that their hue contain more blue and less yellow than the others. Therefore, in that case, in opposite to dairy products, UV barrier seems to protect the product from colour evolution, or at least to slower it.

Graph 12:C* and h° evolution over time at Day 12 and Day 30 and between reference stored in the dark, PP + EVOH exposed to light and PP + EVOH + UV block exposed to light, for almond-based products



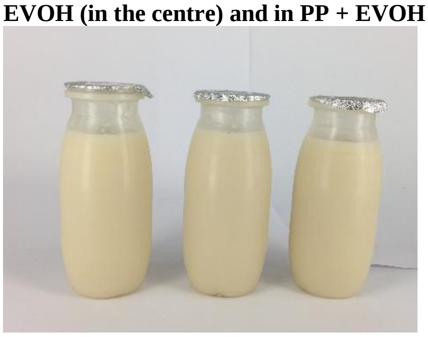
Differences were also noted regarding lightness of the product, on Graph 13.

Graph 13: Evolution of lightness of fermented almond based between Day 12 and Day 30 for reference stored in the dark and PP+EVOH and PP+EVOH+UV block samples exposed to light



For the three options, the product tends to become lighter over time. This effect seems to be accelerated when exposed to light and especially by UV light, since the product exposed to light in a bottle without any UV barrier has a higher level of lightness at day 12 and day 30. This tends to confirm the lipid oxidation observed in gas chromatography, which provoked product's discoloration (Tazi, Plantevin, Di Falco, Puigserver, & Ajandouz, 2009). In the same way as for dairy product, DE was calculated. DE between packaging options is below 1, therefore differences should not be observable, as one can observe on Picture 4. However, evolution over time seems to be perceptible, with a DE above 1.

Picture 4: Fermented almond-based milk in packaging stored in the dark (on the left), in PP + EVOH (in the centre) and in PP + EVOH + UV-block (on the right), at D12



4.2.5.2 Without headspace

Cow milk:

Without headspace, the colour of fermented cow milk is not evolving anymore. The absence of oxygen seems to protect the product from colour degradation, which tends to confirm the hypothesis that lipid oxidation is responsible for colour change, since less oxidation was noticed by gas chromatography as well. The graphs are in Appendix D.

Almond-based product:

Regarding L*, C* and h°, in the same way as for dairy products, there is no difference anymore between packaging options, storage conditions and over time. Picture 5 and Picture 6 show this stability. The graphs are in Appendix E.

Picture 5: Fermented almond-based milk in packaging stored in the dark (on the left), in PP + EVOH (in the centre) and in PP + EVOH + UV-block (on the right), at D30

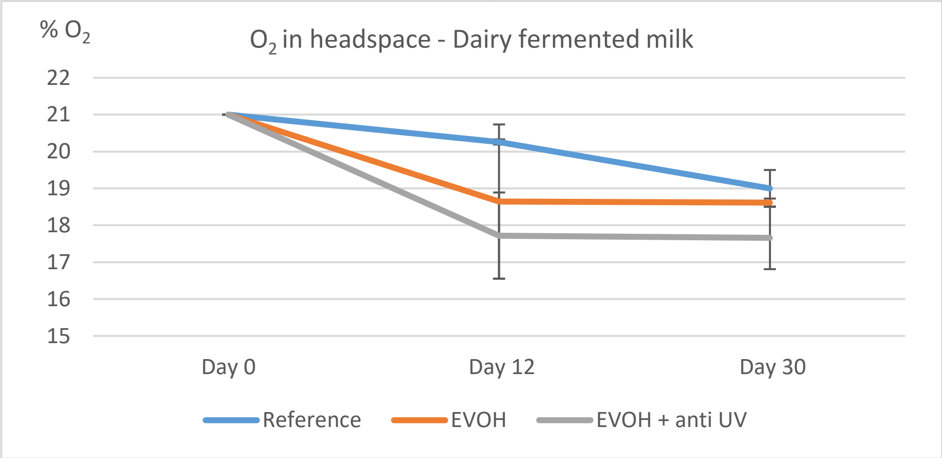
Picture 6: Fermented almond-based milk in packaging stored in the dark (on the left), in PP + EVOH (in the centre) and in PP + EVOH + UV-block (on the right), at D42

4.2.6 **Gas composition in headspace – O_2 and CO_2**

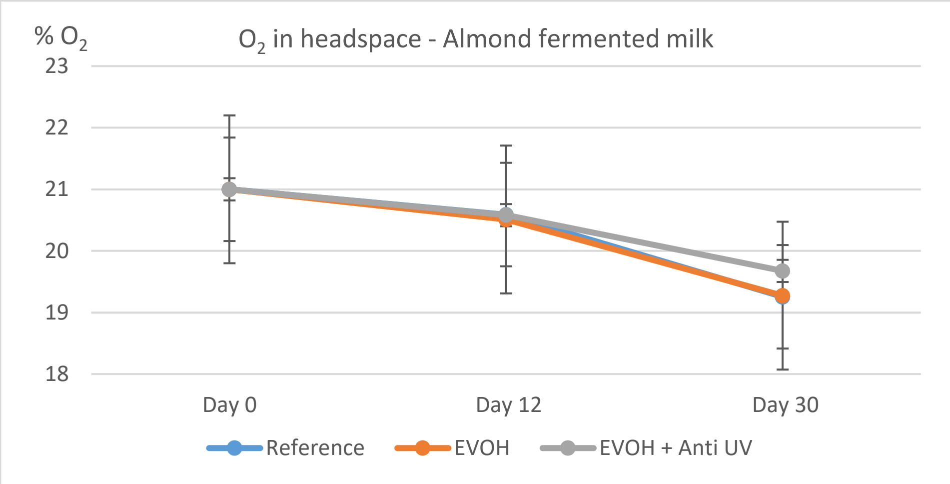
4.2.6.1 With headspace

Graph 14 and Graph 15 show that for both almond and cow fermented milk, oxygen content has decreased in the headspace, and seems to decrease more and faster in the samples exposed to light even though differences are not significant and thus it remains a tendency. This confirms the gas chromatography analysis and sensory test which detected molecules coming from photo-oxidation. Therefore, it confirmed the relevance of making a new light test on products with reduced headspace. However, the decrease being low, it raises the hypothesis that oxidation may also come from oxygen dissolved in the product.

Graph 14: Oxygen evolution in headspace for dairy products exposed to light in PP + EVOH and PP + EVOH + UV block, and for reference product stored in the dark



Graph 15: Oxygen evolution in headspace for almond-based milk products exposed to light in PP + EVOH and PP + EVOH + UV block, and for reference product stored in the dark



4.2.6.2 Without headspace

Since these products did not have any headspace, this measure was not relevant.

Based on all these results, conclusions and designs guidelines were defined and summed up in Chapter 5.

4.3 Case 2 – Fermented milk mixed with fruit and vegetable preparation

Same tests were done on case 2 products, except from gas chromatography.

4.3.1 **Organoleptic evaluation**

No consensus among the participants was observed after nine days, just after light exposure for both products mixed with red and green preparations. Moreover, during the 45 days, no consensus on differences between packaging exposed to light or not was observed.

Red fruit preparation

At the end of shelf-life, all participants could make two groups, based on similitudes. Both samples packed in PP + EVOH were very acidic. This can be explained by two phenomena: ferment X is fermenting malic acid present in the fruit, which creates

CO_2 (McFeeters, Fleming, & Thompson, 1982). In HDPE pack, which is a very poor barrier to gas (see Table 1), part of this CO_2 is released in the atmosphere. In PP + EVOH, CO_2 cannot escape from the pack and therefore is dissolved in the product and provokes its acidification. Another hypothesis comes from the fact that ferment X is anaerobic: in a high barrier to gas packaging, the ferment is in a suitable environment to grow and post-acidify the product, which does not happen in a low barrier to gas packaging, where oxygen may inhibit their activity. In every case, more CO_2 is dissolved in the product when packed in a high barrier packaging compared to a poor barrier one.

After 40 days, at the end of shelf-life + 30%, all products, once again mostly red ones, were very acidic, both those packed in HDPE and PP + EVOH bottles.

<u>Green fruit preparation:</u>

For products with green fruit preparations, all participants also found products packed in PP + EVOH bottles more acidic than the ones packed in HDPE bottles. However, they resent it less acidic than the one with red fruit preparation, and more green fruit and vegetables from the recipe were detected. All participants also felt at day 30 old-cheese, milk and fish off-notes in the products packed in HDPE bottles. Oxidation reactions seem to have occurred in HDPE bottles for green products. One hypothesis can be made from this observation: oxidations may have also happened in products made of red fruit preparation, however the high acidity may hide the off-notes. This may be confirmed or disproved by the oxygen content measured in the headspace.

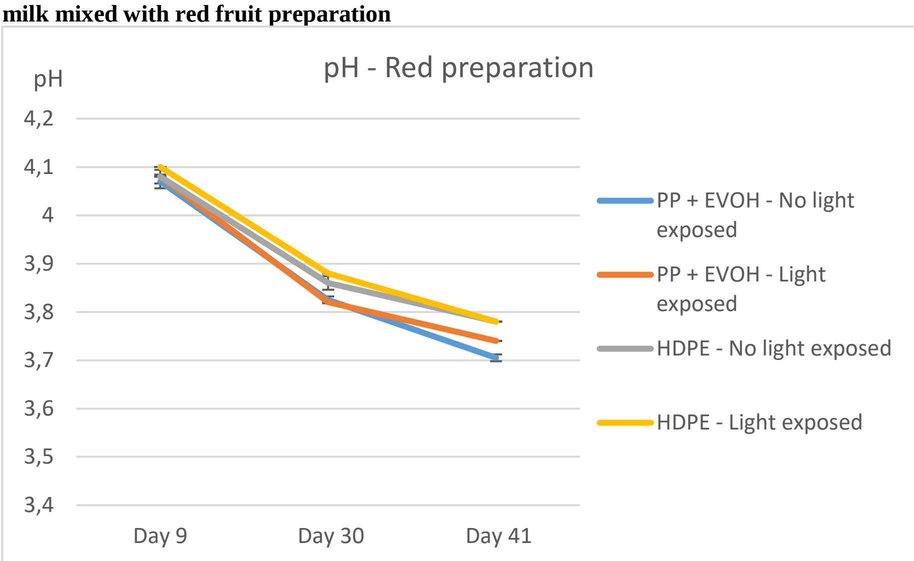
4.3.2 **pH measurement**

Graph 16 and Graph 17 show a decrease in pH for all options by 0,4 points at maximum. Moreover, significant differences between packaging options were noticed in the red fruit preparation though.

<u>Red fruit preparation</u>

For the red fruit preparation, a significant difference was observed between the samples packed in PP + EVOH and those packed in HDPE. In accordance with what was observed during the organoleptic evaluation, the pH dropped more in products in PP + EVOH bottles than in HDPE bottles. Moreover, a slightly lower pH was observed at the end of the test in the HDPE bottles stored in the dark. No significant differences between products exposed to light and stored in the dark was observed.

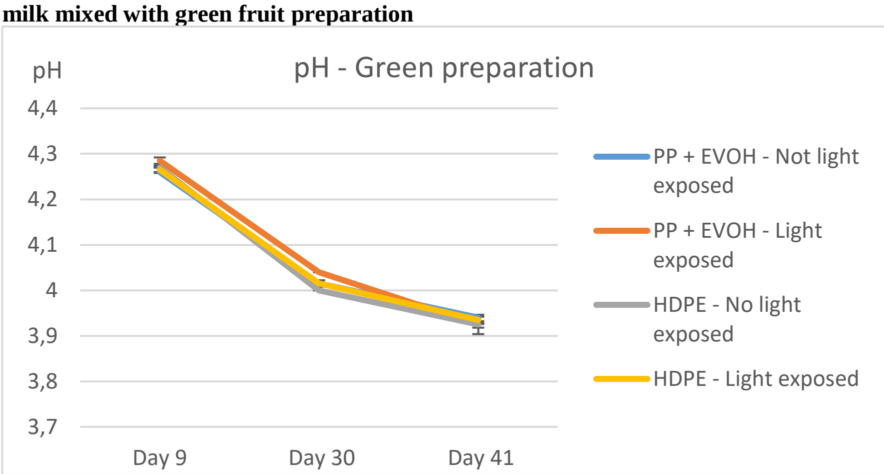
Graph 16: pH evolution for both packaging and both storage conditions for fermented cow milk mixed with red fruit preparation



<u>Green fruit preparation:</u>

For product made of green fruit preparation, no significant differences were observed between pack and storage conditions. pH decreased in all samples.

Graph 17: pH evolution for both packaging and both storage conditions for fermented cow milk mixed with green fruit preparation

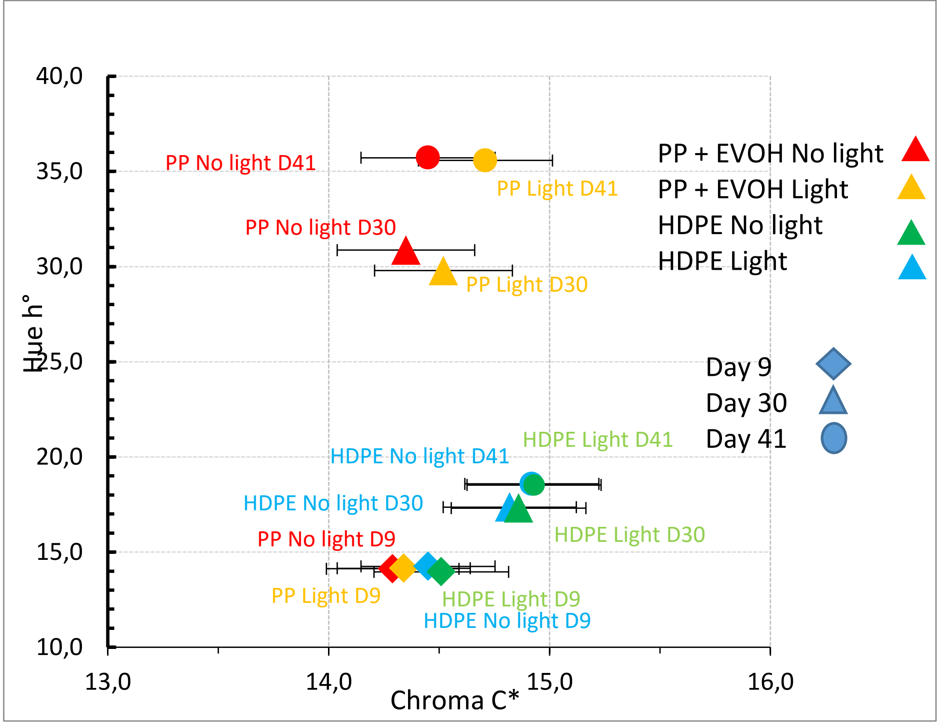


4.3.3 **Colour measurement**

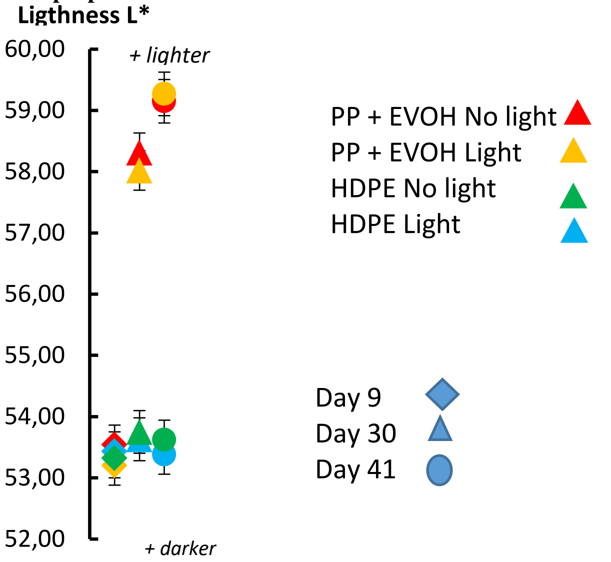
<u>Red fruit preparation</u>

Graph 18 shows significant differences regarding the parameter h° at day 30 and day 41 between packaging options mostly, and between samples exposed to light or not as well. Fermented cow milk with red fruit preparation packed in PP + EVOH results in having more yellow than the samples packed in HDPE. Moreover, for both packaging materials, products exposed to light seem to have a slightly higher chroma, thus to be more vivid than the ones stayed in the dark. But this difference, although observed for every packaging at every test, does not seem to be significative.

Graph 18: C* and h° evolution for fermented cow milk mixed with red fruit preparation at Day 9, Day 30 and Day 41



Graph 19: L* evolution at Day 9, Day 30 and Day 41 for fermented cow milk mixed with red fruit preparation



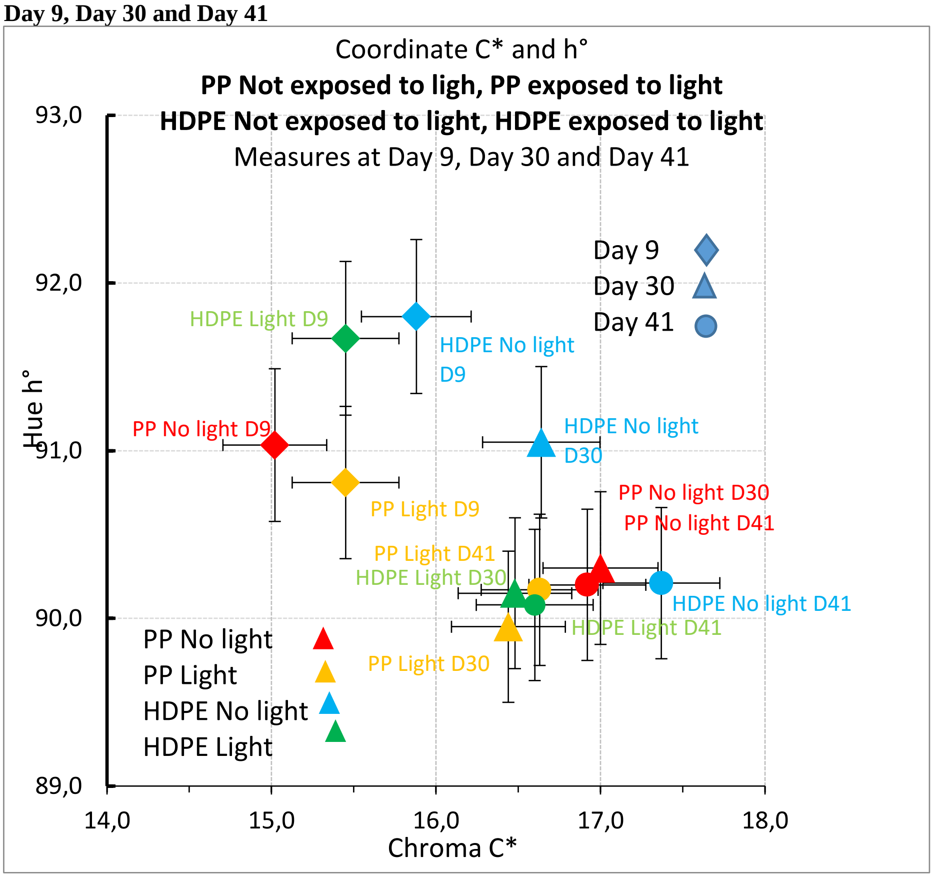
DE was calculated to define if these significative differences were perceptible to the naked eye. The evolution over time is perceptible, with a DE between 1 and 5 between samples at day 9 and between samples at day 41. Moreover, between samples packed in HDPE and samples packed in PP bottles, DE is higher than 5, meaning that the two colours are totally different, as seen on Picture 7. However, between samples packed in the same material exposed to light or stored in the dark, DE is smaller than 1: the differences are significantly different but not perceptible to the naked eye. Therefore, oxygen alone seems to have an impact on the colour, without considerable influence of light. OTR of the material chosen will thus be a key factor to consider.

Picture 7: Fermented cow milk mixed with red fruit preparation at Day 30 (on left) and at Day 60 (on right). From left to right on each picture: HDPE not exposed to light, HDPE exposed to light, PP + EVOH not exposed to light, PP + EVOH exposed to light

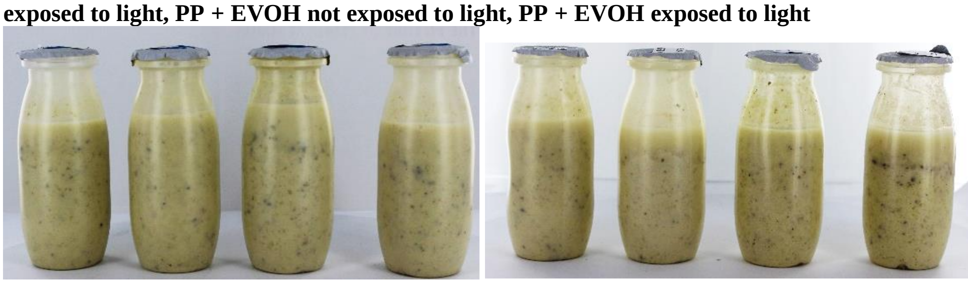
<u>Green fruit preparation</u>

For green fruit preparation, no significant differences were observed between packaging options and storage conditions. However, as shown on Graph 20 for all options, C* is increasing between Day 9 and Day 30. Thus, products are getting more vivid with time. No difference was observed between samples exposed to light and stored in the dark.

Graph 20:C* and h° evolution for fermented cow milk mixed with green fruit preparation at Day 9, Day 30 and Day 41



Picture 8: Fermented cow milk mixed with green fruit preparation at Day 9 (on left) and at Day 30 (on right). From left to right on each picture: HDPE not exposed to light, HDPE exposed to light, PP + EVOH not exposed to light, PP + EVOH exposed to light

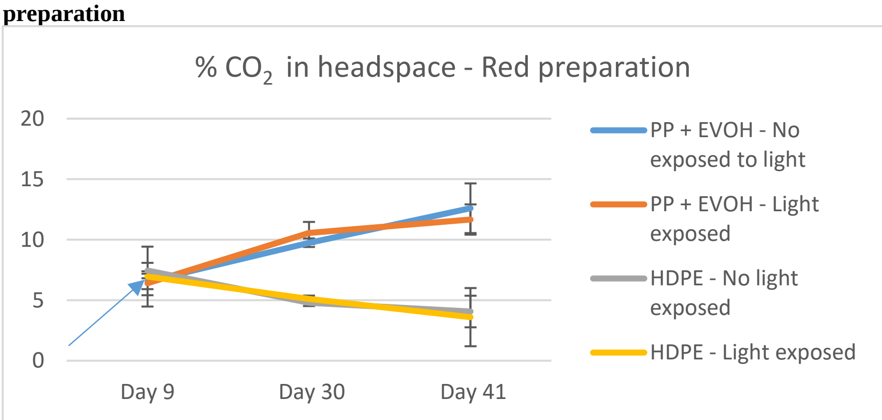


4.3.4 **Headspace gas composition – O_2 and CO_2**

Red fruit preparation:

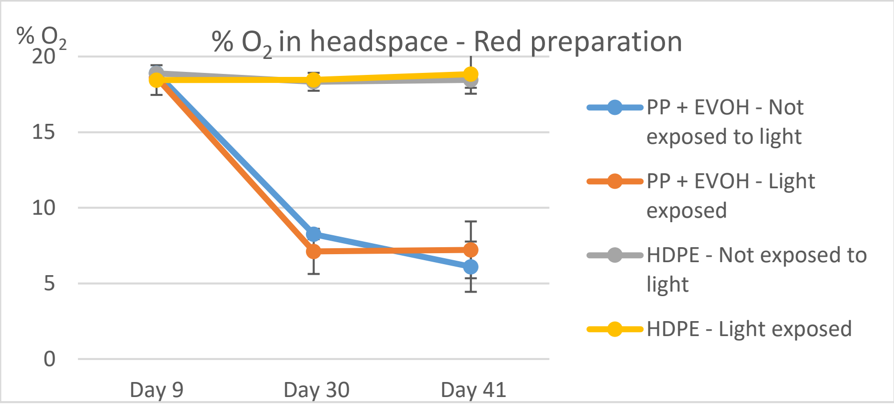
The measurement of O_2 and CO_2 helped explain the previous observations. Graph 21 shows that CO_2 content is the same in every product at day 9, matching the fact that no difference between samples was observed in sensory tests. However, this content doubled between day 9 and day 45 in the red products packed in PP + EVOH bottles, whereas it decreased in red products packed in HDPE bottles. It can be assumed that between day 0 and 9, the speed at which CO_2 is produced is higher than the CO_2 transmission rate through HDPE bottles. Therefore, there is no differences between products packed in dissimilar materials. However, between 9 and 41 days, the speed of CO_2 production becomes slower, and CO_2 can escape from HDPE bottles, whereas it is stuck in PP bottles, where its amount keeps increasing. Part of this CO_2 may be dissolved in the product, in a higher amount in PP bottles since none of it can escape from these bottles, and cause the decrease of pH and acidity felt in the mouth.

Graph 21: CO_2 content evolution in headspace for fermented cow milk with red fruit preparation



Furthermore, Graph 22 shows that oxygen level is falling down in the products packed in PP + EVOH bottles, while remaining constant in the products packed in HDPE. No difference or oxidation off-notes in sensory test was observed. However, the important acidity may have hidden all these off-notes, as explained in 4.3.1. A new hypothesis which can be made at this stage is that the same amount of oxygen may be consumed by all products, but HDPE bottles being gas permeable, oxygen from the environment enters the bottle and replaces the oxygen which has been consumed, maintaining a constant amount of oxygen in the headspace. Indeed, there is no argument supporting a higher oxidation in high gas barrier packaging, and the contrary is more likely to happen. Once again, no significant difference was observed on these parameters, both oxygen and carbon dioxide, between samples exposed under light and samples kept in the dark.

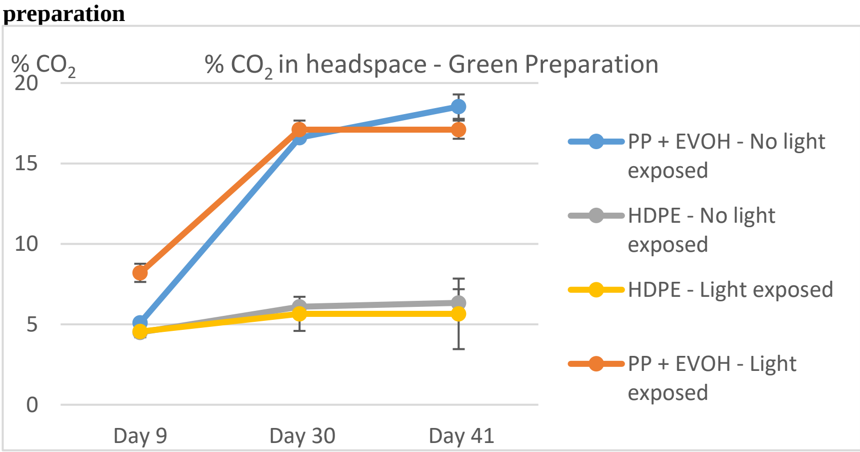
Graph 22: O_2 content evolution in headspace for fermented cow milk with red fruit preparation



Green fruit preparation

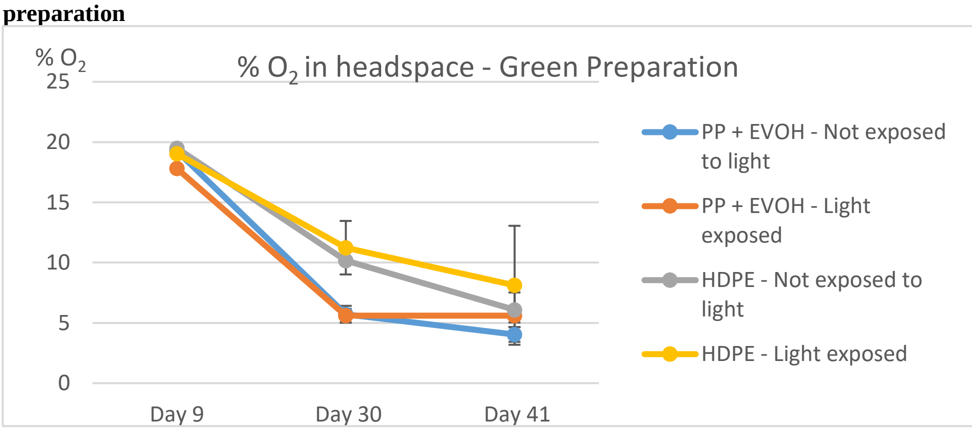
Graph 23 shows that CO_2 increases a lot in the samples packed in PP + EVOH bottles, whereas it stays stable in the samples packed in HDPE bottles. The final amount of carbon dioxide at day 45 in PP + EVOH bottles is much higher with green fruit preparation than with red one. Therefore, it can be assumed that more CO_2 is produced and thus, the speed of production between 9 and 45 days is equivalent to the HDPE gas transmission rate. Another hypothesis can be deduced from the sensory test and acidity measures. Green products are less acidic than red products. It can be supposed that the same amount or even more of CO_2 is produced in green fruit products, but this gas may be more soluble in red fruit preparation than in the green one. Therefore, more gas would stay in the headspace in green products, without acidifying the mass.

Graph 23: CO_2 content evolution in headspace for fermented cow milk with green fruit preparation



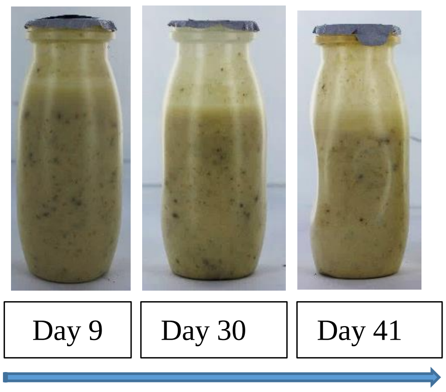
On Graph 24, O_2 content decreases in all packaging options between 9 and 45 days. After 30 days though, the amount of O_2 is much lower in PP + EVOH bottles than in HDPE ones. It confirms the hypothesis that oxygen from the environment may replace the oxygen consumed in bottles made of poor barrier material. However, compared to red fruit products, O_2 consumption in green products seems to be faster than HDPE rate. What can be concluded, and in accordance with the “old cheese” off-notes described during organoleptic evaluation is that oxidation reactions happen in the product during storage. Graph 24 highlights that oxygen content stays constant between 30 and 41 days. The concentration may have reached a too low threshold, making oxidation reactions stop. Old cheese and fish off-notes were only observed in the products packed in HDPE products. Thus, it can be assumed that more oxidation reactions happen in this pack, since the source of O_2 is higher, the gas consumed being replaced by gas from the environment.

Graph 24: O_2 content evolution in headspace for fermented cow milk with green fruit preparation



Picture 9 illustrates the higher speed of oxygen consumption by green fruit preparation products compared to HDPE’s OTR, provoking vacuum inside the bottle, and therefore a bottle squeeze, which may impact FMOT for consumers. As for products made of red fruit preparation, no difference was observed between samples exposed to light and samples stored in the dark.

Picture 9: HDPE bottle evolution over time with green fruit preparation



5 Conclusions and further research

The three main goals of this master thesis were tackled. The conclusions of each of them as well as discussion about the results are presented in the following subchapters. One needs to remind that these results are very dependent on the food matrix which was studied, and a new light test on a new food matrix, with different ferments, other content in fats vitamins or any other sensitive component may give different results. This thesis gives a detailed method and protocol to study the impact of light and oxygen, which can be adapted to every food matrix and packaging. Therefore, the results exposed in this thesis should not be taken as a general truth, but as an example of how light and oxygen impact can be evaluated. Finally, some tracks for further research are exposed.

5.1 New light test protocol

Based on the current light test protocol used in Danone Research Centre in Palaiseau, a new one was elaborated, based on two main stages in product and packaging development process. A test "UNDERSTAND" was elaborated to study in a scientific way the impact of light and oxygen on product's stability, at the early stage of the development, to get insight on transparency accessibility for the packaging. A test "VALIDATE" was elaborated for later stages in the process, once the final product and packaging are developed, to check if the product performs as expected in real supermarket conditions. Moreover, two kinds of light, LED and fluorescent were recommended to test, knowing that in many countries, like France, there is a fast change towards LED technology. Finally, a base of analytical methods was also established, to correlate the observations from sensory test with chemical mechanisms, and understand better how light and oxygen impact the product's quality. It would help give more accurate design guidelines for packaging developers.

5.2 Light and oxygen impact on products

5.2.1 **Case 1**

Light has shown to have a significant effect on the organoleptic properties of both fermented cow and almond milks. Rancid, carboard, old cheese off-notes were detected in fermented cow milks exposed to light and cardboard and rancid off-notes were perceived in fermented almond milk. An important learning from Case 1 results, is that visible light seems to play a more significant role in photo-oxidation of such products than UV wavelengths. It confirms that chlorophylls and porphyrins oxidation is more correlated to sensory defects than riboflavin degradation, as it is more commonly said. Colour, mostly chroma, of the products are also evolving under light, while remaining stable when the products are stored in the dark. Removing the headspace, simulating an azote flushing, helped improve the product properties against light exposure. The colour is not evolving anymore, and less photo-oxidation and oxidation reactions occur, as observed during sensory test and confirmed by the gas chromatography analysis. Off-notes are still perceived without headspace, even though in less quantities: a consumer acceptance test would tell the developers if transparency is accessible or not. Finally, the product's pH stays stable under light or in the dark, with headspace or without.

One results remains difficult to explain in this test. UV block, for organoleptic properties as well as for colour in dairy products, seems to enhance product's evolution during storage. Even though visible light seems to have a major impact on photo-oxidation in these products, the fact that UV block makes it worst remains unexplained. A further study on the UV block used in this thesis may be interesting in order to fully understand these results.

5.2.2 **Case 2**

Case 2 on fermented cow milk mixed with 40% of fruit preparations clearly showed two aspects: the first one regarding packaging permeability effect, and the other one regarding light impact. First, almost every fruit has a high content in malic acid. Therefore, when mixed with a ferment which is still alive and can ferment malic acid and generate CO_2 as in Case 2, packaging material permeability becomes a very important asset which needs to be looked at. Indeed, if it cannot escape from the packaging, CO_2 may behave in two ways, which will affect product's quality and/or consumer perception: it can stay in the headspace and deform the lid, creating a bulge, or part of it can be dissolved within the product, leading to a post-acidification of the product. In fermented products where gases are produced, it is thus advised to use packaging material with a poor barrier to gas. However, if such materials are used, another risk arouses. Off-notes such as cheese and fish, were observed after

30 days in green products packed in HDPE bottles. These off-notes are typical photo-oxidation markers. Full transparency may thus not be accessible for some products, depending on the matrix. These off-notes were not observed in red matrixes. Two hypotheses can be made at this stage: red products contain many anthocyanins pigments, which have anti-oxidant properties, so they may have protected the product against oxidation. Or the acidity of the product after 30 days hided all the off-notes. Finally, an important parameter to follow for these kind of products is the colour stability. Pigments, such as anthocyanins and carotenoids, can be degraded by light, or change colouration according to the pH. Therefore, in products where pH is decreasing over the shelf-life, such as fermented products and more specifically in products from Case 2, pigments characteristics should be carefully studied, in order to maintain an acceptable colour all over the product's shelf-life.

5.3 Design guidelines for each product

5.3.1 **Case 1 - Fermented cow and plant-based milks**

What can be deduced from the tests was that visible light seems to affect in a more significant way the product than UV wavelengths. Indeed, products packed in a bottle with UV block did not give better results than products packed without. It even gave worst results, which might come from the UV agent used for the tests. Therefore, UV barrier does not seem necessary for these products. Furthermore, removing the headspace gave better results regarding sensory tests and aromatic profiles. **To have full transparency, a packaging with a high oxygen barrier, using EVOH layer, seems to be necessary, and having N_2 in the headspace seems to improve the product's quality**, preventing from part of the off-notes. **If a consumer test shows that the off-notes remaining can be noticed, full transparency may be not accessible.** Further tests need to be done to confirm all these first design guidelines, with an industrial filling for instance, to remove the dissolved oxygen which may have entered the product during manual filling. This may remove all off-notes observed in the test without any headspace, since it would decrease even more the oxygen available.

5.3.2 **Case 2 – Fermented cow milk mixed with fruit and vegetable preparation**

A first guideline is that at least partial transparency seems accessible for these products. Indeed, no significant difference was observed between the samples exposed and not exposed to the dark. However, they all had a UV block, which may have partly protected the products. Thus, UV block may be necessary to reach transparency. Some off-notes were detected in green products. Therefore, depending on the matrix, full transparency or partial transparency may be reached. Regarding the packaging material permeability, a material which lets the gas escape seems to be a better option: CO_2 is produced via malic acid fermentation and has a consequent impact on product's organoleptic properties and pH. However, HDPE proved to be too weak in the green product case, where oxygen is consumed very fast, which create vacuum inside and a squeeze of the bottle. Adding material to have a thicker HDPE bottle may be a solution. A **thick HDPE bottle with UV-block** seems to be a good option for these products, with **full or partial transparency** depending on the fruits and vegetables.

5.4 Further research

Further research can be done on this topic and may be classified into four categories.

The impact of the type of light, fluorescent tubes and LED could be studied, to see if the products are less sensitive to LED, which do not emit in UV and do not produce heat while illuminating.

More packaging barriers may be tested: Case 1 products have not been tested in packaging without any oxygen barrier. In the same way, Case 2 products could be tested without any UV barrier, since it has shown in the Case 1 to be unnecessary.

The main problem in the products in Case 2 was the fermentation of malic acid, which leads to a production of CO_2 and a decrease of pH and thus lower organoleptic properties and deformation of the packaging. A change of recipe may be done to have fruits with a lower content in malic acid, or ferments which do not react with it.

Finally, two more following-up may be done during the light tests which will be done next. Bacteria counting would help know better the ferments activity and thus the impact of light on it. Indeed, if pH does not evolve, this means that ferments do not post-ferment, however it does not give any information on their state: if light make them inactive, or if light kills them. Based on the results, companies may not be able to claim the same on their products. Another interesting test which may be done is the follow-up of the dissolved oxygen in the product. A first hypothesis was made, saying that the main source of oxygen leading to photo-oxygen reactions was the oxygen in the headspace. However, even though a reduced headspace improves the organoleptic properties of the products in case 1, some off-notes were still perceived when the products were exposed to the light. The products being made manually in a pilot, more oxygen than in industrial conditions may be stuck in the product during conditioning, and may enhance photo-oxidation. This might not happen in real life, when products are made in factories.

6 Bibliography

Becker, E., Christensen, J., Frederiksen, C., & Haugaard, V. (2003). Front-Face Fluorescence Spectroscopy and Chemometrics in Analysis of Yogurt: Rapid Analysis of Riboflavin. *Journal of Dairy Science*, 85:2508-2515.

Bekbölet, M. (1990). Light Effects on Food. *Journal of Food Protection*, 430-440, Vol. 53, No.5.

Bell, T., Alamzad, R., & Graf, B. (2016). Effect of pH on the chemical stability of carotenoids in juice. *New Technology in nutrition research and practice*.

Borari Lima, A., Duarte Correa, A., Aparecida Saczk, A., Pereira Martins, M., & Oliveira Castilho, R. (2003). Anthocyanins, piments stability and antioxidant activity in jabuticaba. *Revista Brasileira de Frucultura*, 877-887.

Borle, F., Sieber, R., & Bosset J.O. (2011). Photo-oxidation and photoprotection of foods, with particular reference to dairy products - An update of a review article . *Science des Aliments, Vol. 21*, 571-590.

Bothersen, C., McMahon, D., Legako, J., & Martini, S. (2016). Comparison of milk oxidation by exposure to LED and fluorescent light. *Journal of Dairy Science*, 99:2537-2544.

Cakmakçi, S., & Turgut, T. (2005). Influence of Different Sources, Illumination Intensities and Storage Times on the Vitamin C Content in Pasteurized Milk. *Turkish Journal of Veterinary and Animal Sciences*, 1097-1100.

Christophersen, A., Bertelsen, G., Andersen, H., Knuthsen, P., & Skibsted, L. (1992). Storage life of frozen salmonoids - Effect of light and packaging conditions on carotenoid oxidation and lipid oxidation. *Z Lebensm Unters Forsch*, 115-119.

Corner, & Paine. (2002). Chapter 8, The Special Worlds of Packaging and Marketing.

Decker, E., Elias, R., & McClements, D. (2010). Oxidation in foods and beverages and antioxidant applications, Understanding mechanisms of oxidation and antioxidant activity. Dans *Vol.1. 978-1-84569-648-1* (pp. 66-67). Cambridge: Woodhead Publishing Limited.

Domingos, L., Xavier, A., Mercadante, A., Petenate, A., Jorge, R., & Viotto, W. (2014). Oxidative stability of yogurt with added lutein dye. *Journal of Dairy Science*, 97:616-23.

Duncan, S., van Aardt, M., & Marcy, J. (2001). Effectiveness of Poly(ethylene terephtalate) and High-Density Polyethylene in Protection of Milk Flavor. *Journal of Dairy Science*, 84:1341-1347.

Elbandy, M., & Abdelfadeil, M. (2008). Stability of Betalain Pigments from Red Beetroot (Beta vulgaris). *Egyptian Journal of Food Science*, 49-60.

Frankel, E. (1980). Lipid oxidation. *Progress in Lipid Research Vol 19*, pp. 1-22.

Frederiksen, C., Haugaard, V. K., Poll, L., & Becker, E. M. (2003). Ligh-induced quality changes in plain yoghurt packed in polylactate and polystyrene. *European Food Research Technology*, 217:61-69.

Gustafsson, J. (2017). *Single case sutdy vs. multiple case studies: A comparative study.* Halmstad, Sweden: Academy og Business, Engineering and Science.

Hornedo-Ortega, R., Alvarez-Fernandez, A., Cerezo, A., Troncoso, A., & Garcia-Parilla, M. (2016). Influence of storage conditions on the anthocyanin profile and colour of an innovative beverage elaborated by gluconic fermentation of strawberry . *Nutrition and Food Science*, 198-209.

Intawiwat, N. (2011). *Minimizing photooxidation in dairy products by tailor made light barrier properties in packaging materials.* Dept. of Chemistry, Biotechnology and Food Science - Norwegian University of Life Science.

Intawiwat, N., Pettersen, M., Rukke, E., Meier, M., Vogt, G., Dahl, A., . . . Wold, J. (2009). Effect of different colored filters on photooxidation in pasteurized milk. *Journal of Dairy Science*, 1372-1382.

Johnson, D., Duncan, S., Bianchi, L., Chang, H., Eigel, W., & O'Keefe, S. (2015). Packaging modifications for protecting flavor of extended-shelf-life milk from light. *Journal of Dairy Science*, 98:2205-2014.

Klewicka, E. (2010). Fermented Beetroot Juice as a Factor Limiting Chemical Mutations Induced vy MNNG in Salmonella typhimurium TA98 and TA100 Strains. *Food Technology Biotechnology*, 229-233.

Koyuncu, M., & Tuncturk, Y. (2017). Effect of packaging method and light exposure on oxidation and lipolysis in butter. *Oxidation Communications 40, No 2*, 785-798.

Larsen, H., Geiner Tellefsen, S. B., & Dahl, A. (2009). Quality of sour cream packaged in cups with different light barrier properties measured by fluorescence spectroscopy and sensory analysis. *Journal of Food Science, Vol. 74, Nr 8*, S345-350.

Lavoie, J.-C., Chessex, P., Rouleau, T., Migneault, D., & Comte, B. (2004). Light-Induced Byproducts of Vitamin C in Multivitamin Solutions. *Clinical Chemistry*, 134-140.

Lennersten, M. (1995). *The Influence of Light and Packaging Materials on Oxidative Deterioration in Foods*. Gothenburg, Sweden: Department of Food Science, Chalmers University of Technology.

Lockamy, A. (1995). A Conceptual Framewrok for Assessing Strategic Packaging Decisions. *The International Journal of Logistics Management*, 51-60, Vol 6. Issue 1.

Löfgren, M., & Witell, L. (2005). Kano's Theory of Attractive Quality and Packaging. *Quality Management Journal*, 7-20.

Marchal, L., & Credoz, Y. (2018, February 8). Ferments with light and O2. (E. Pignères, & F. Chivrac, Intervieweurs)

Marsh, K., & Bugusu, B. (2008). Food Packaging - Roles, Materials and Environmental Issues. *Journal of Food Science, Vol 72, Nr3*.

Mauro, C. S., Guergoletto, K. B., & Garcia, S. (2016). Development of Blueberry and Carrot Juice Blend Fermented By Lactobacillus reuteri LR92. *Beverages*.

McFeeters, R., Fleming, H., & Thompson, R. (1982). Malic Acid as a Source of Carbon Dioxide in Cucumber Juice Fermentation. *Journal of Food Science*.

Mestdagh, F. (2005). Protective influence of several packaging materlas on light oxidation of milk. *Journal of Dairy Science, Vol. 88*, 499-510.

Mortensen, G., Sorensen, J., & Stapelfeldt, H. (2003). Effect of modified atmoshpere packaging and storage conditions on photooxidation pof sliced Havati cheese. *European Food Research and Technology*, 93-98.

Papachristou, C., Badeka, A., Chouliara, E., Kondyli, E., Athanousoulas, A., & Kontominas, M. (2006). Evaluation of polyethylene terephtalate as a packaging material from premium quality whole pasterized milk in Greece. *European Food Research and Technology*, 223:711-718.

Potts, H., Amin, K., & Duncan, S. (2016). Retail lighting and packaging influence consumer acceptance of fluid milk. *Journal of Dairy Science*, 100:146-156.

Profir, A., & Vizireanu, C. (2013). Evolution of antioxidant capacity of blend juice made from beetroot, carrot and celery during refrigerated storage. *The Annals of the University Dunarea de Jos of Galati Fascicle VI - Food Technology*, 93-99.

Roberston, G. (2010). Chapter 7 - Packaging and food and beverage shelf-life. Dans G. Robertson, *Food and Packaging Shelf-life* (pp. 244-269).

Robertson, G. (2010). Chapter 1 - Food Packaging and Shelf-Life. Dans G. Robertson, *Food Packaging and Shelf-Life - A Practical Gudie* (pp. 1-14). Brisbane: University of Queensland and Food-Packaging-Environment.

Sattar, A., & Deman, J. (1975). Photooxidation of milk and dairy products: a review. *Critical Reviews in Food Science and Nutrition*, 14-33.

Sheraz, M. (2014). Photo, thermal and chemical degradation of riboflavin. *Beilstein Journal of Organic Chemistry, Vol.10*, 1999-2012.

Simmonds, G., Woods, A. T., & Spencer, C. (2018). 'Show me your goods': Assessing the effectiveness of transparent packaging vs. product imagery on product evaluation. *Food Quality and Preference*, 18-27.

Smart Light Engineering. (s.d.). *Smart Light Retail & Presentation.* Dojc, Slovakia.

Soarnol. (s.d.). *Gas Barrier Seminar*. Récupéré sur High gas-barrier resin Soarnol: http://www.soarnol.com/eng/solution/solution050723.html

Tazi, S., Plantevin, F., Di Falco, C., Puigserver, A., & Ajandouz, E. (2009). Effects of light, temperature and water activity on the kinetics of lipoxidation in almond-based products. *Food Chemistry*, 958-964.

Van Aardt, M. (2000). Flavor threshold for acetaldehydes in milk, chocolate milk, and srping water using solid phase microextraction gas chromatography for quantification. *Journal of Agriculture and Food Chemistry*, 49(3).

Wold, J., Veberg, A., Nilsen, A., Iani, V., Juzenas, P., & Moan, J. (2005). The role of naturally occurring chlorophyll and porphyrins in light-induced oxidation of dairy products. A study based on fluorescence spectroscopy and sensory analysis. *International Dairy Journal*, 343-353.

Zardin, E., Silock, P., Siefarth, C., & Bremer, P. (2016). Dynamic changes in the volatiles and sensory properties of chilled milk during exposure to light. *International Dairy Journal*, 35-38.

Appendix A – Organoleptic evaluation questionnaire for Case 1

0	1	2	3	4	5
Absence	Very weak	Weak	Mean	Strong	Very strong

NAME
DATE

	Texture	**Odour**		**Taste**		**Aromatic Notes**				
Code	**Thickness** 0: not thick at all 5: extremely thick	**Off-flavour** intensity	**Off-flavour** identification	**Sweetness** intensity	**Acidity** Intensity	**Milky** intensity	**Off flavors** intensity	**Off flavors** identification	**Aftertaste** length	**Comments**

Appendix B – Organoleptic evaluation questionnaire for Case 2

Compare 4 references (GREEN) and 4 references (RED)

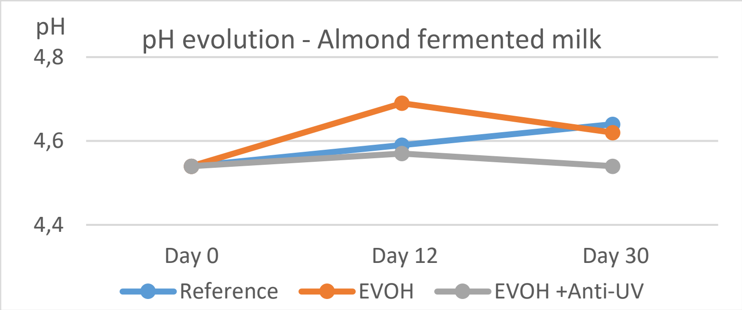
Name

Date

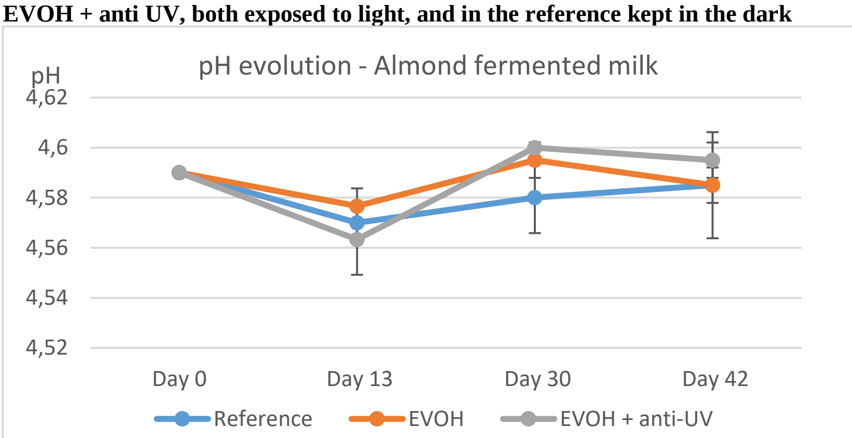
	Smell	Taste	Taste lasting	
Sample Code	**Off-notes**	**Off-notes**	**Off notes**	**Comments**
Smell and taste Green product bottle and give your preference				
Smell and taste Red product bottle and give your preference				

Appendix C – Graphs of pH evolution for almond based products

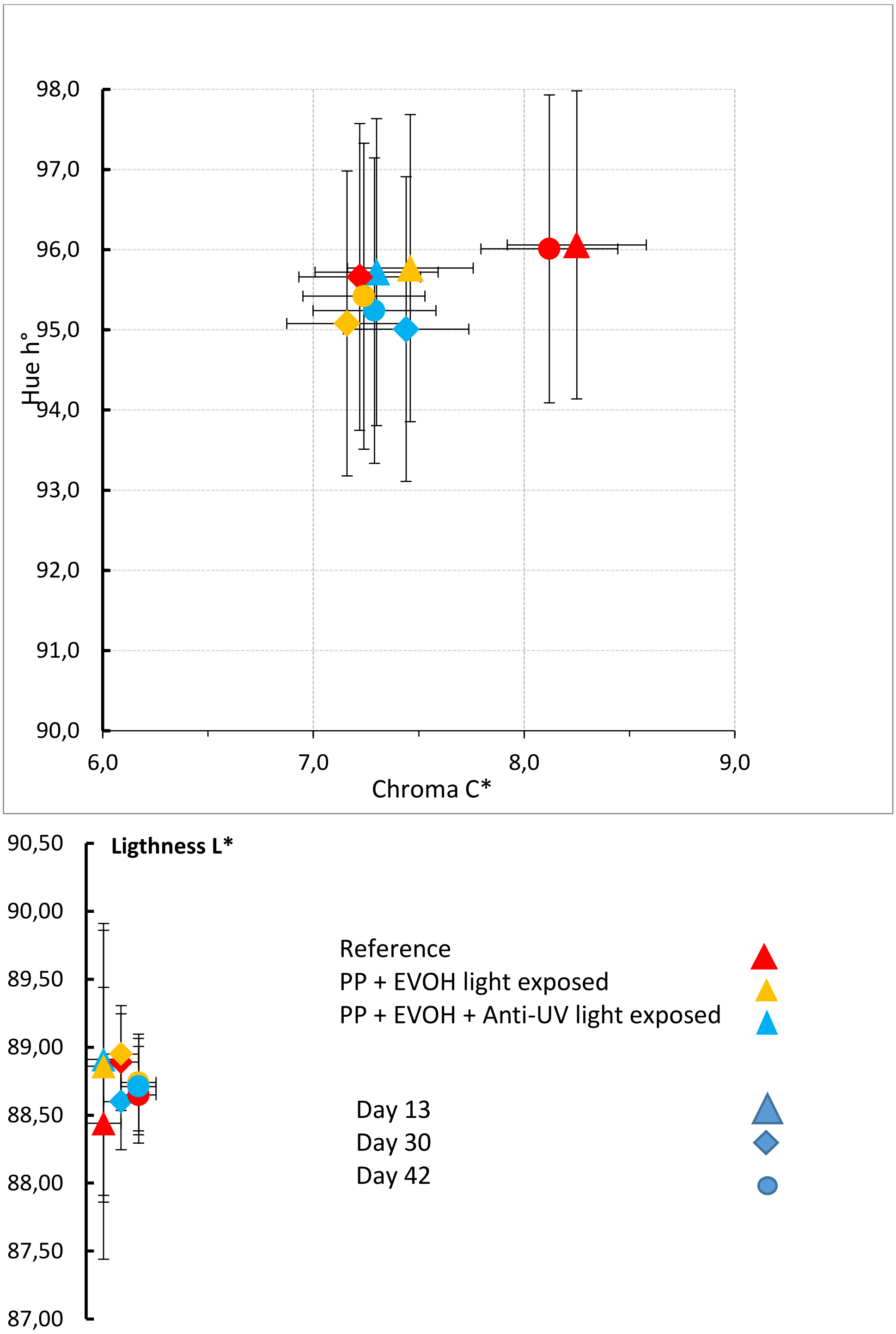
pH evolution for almond-based products packed in PP + EVOH, PP + EVOH + anti UV exposed to light, and in the reference kept in the dark



pH evolution for almond-based products packed without headspace in PP + EVOH, PP + EVOH + anti UV, both exposed to light, and in the reference kept in the dark



Appendix D – Colour evolution of fermented cow milk without headspace



Appendix E – Colour evolution for fermented almond milk without headspace