Sensory evaluation and quality assessment of an alternative inner coating film in yogurt cartons

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Thesis work done at Arla Foods Linköping
2015-01-12 – 2015-05-29

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The study was based on the analysis and measurement of sensory attributes performed by experts, physical properties in laboratory and chemical composition in GC-FID/MS together with a discriminative test where a group of people would identify any difference between the yogurts. Together, these analyses would provide an explanation about any differences between the packaging materials by connecting physical, chemical and/or sensory characteristics. The collected results would give a better and more comprehensive picture than each analysis would do separately.

The results from the study show that there is a difference between yogurts stored in LDPE-based containers and yogurts stored in EVOH-based containers and that the product was chemically affected, mainly by the level of oxygen in contact with the food. The overall assessment is that the largest difference was discovered in the taste.
Abstract

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Abbriveations

- LDPE – Low-density Polyethylene
- EVOH – Ethylene Vinyl-alcohol
- OTR – Oxygen Transmission Rate
- GC – Gas Chromatography
- MS – Mass Spectrometry
- FID – Flame Ionisation Detection
- DHS – Dynamic Headspace Sampling
- CFU – Colony Forming Units
1. Introduction

Arla Foods is a manufacturer and exporter of different dairy products all around the world. From an estimated 210,000 tonnes of milk, the factory in Linköping manufactures a wide range of special dairy products such as lactose free milk, yogurt, crème fraiche etc. Approximately 50% of the production at Linköping dairy is directed to the production of yogurt which is distributed to food-chains and customers all around Sweden. The company is a cooperative owned by 13,500 dairy farmers from all around Europe. Their principle is to work together to “continuously create something stronger and better, for the benefit of our farmer owners”. Arla works hard to keep the business sustainable by close cooperation with the customers and collaborators, and strives to work with responsibility right from the farm, through production and all the way to recycling in order to minimize their environmental impact. Arla Foods has 19,600 employees in 30 countries worldwide and a revenue of 10.6 billion EUR by the year 2014. (Arla Foods AB, 2015)

Food packaging has been a costly and demanding area in the company which puts high requirements on both staff and equipment. The packaging process is sensitive and a small stop or disturbance in the packaging line can lead to a noticeable delay in the schedule. The society today also put on high requirements on the packaging material in terms of protecting its content by being a barrier against moisture, light, gases and water. Some anaerobic products are specifically dependant on being protected against oxygen to keep its characteristic taste, smell and colour. Dairy products are known to undergo oxidation during storage, resulting in a deterioration of sensory properties such as smell and taste. Specifically milk is prone to be influenced by oxidation due to its high content of unsaturated milk fats (Hedegaard et al. 2006). A previous study has been made on the packaging material of milk where oxidation is identified as the main cause for a sensory deterioration (Refthammar M. 2015). To achieve protection against oxygen in the packaging, food companies such as Arla uses co-extruded, multi-layered plastic films of low-density polyethylene (LDPE) and ethylene vinyl alcohol (EVOH), forming a strong barrier material to protect its contents.

Complex, multi-layered barrier materials such as EVOH is costly and relatively difficult to manufacture compared to single-layered LDPE materials. Arla Foods is currently investigating the possibility of changing the packaging material, from the multi-layered EVOH to the single-layered LDPE, in some of their dairy products. The previous study by Refthammar found that a change from EVOH- to LDPE-coated packaging material of milk resulted in a deterioration of the milk. It would not likely be accepted in the public and Arla Foods decided to investigate further and try it on other products. The company want to investigate how changing the packaging material affects dairy products with different properties than that of milk. This study is the second in line and aims to investigate the possibility of replacing the existing EVOH-based coating of yogurt cartons with the less expensive and simpler LDPE without significantly affecting any of the organoleptic properties (taste, smell, sight etc.), quality or shelf-life of the yogurt. It is believed that yogurt, being a different product than milk will react differently and hopefully remain relatively unaffected to a change in packaging material. This study is designed to investigate the interaction between the product and the packaging material during storage by measuring and analysing chemical, physical and sensory changes.

1.1. Purpose of the study

Co-extruded multi-layer plastics (explained in section 3.1.) are becoming increasingly common for use in packaging applications for food and dairy products thanks to their combined properties making them ideal as food carriers. Plastic packaging materials have always been subject to questioning
regarding their performance in terms of permeability and migration of substances that jeopardises the safety and quality of the product. While consumer safety of the products is arguably the most important factor for food companies, the organoleptic changes that occur in plastic packaged food is almost of equal importance. The main focus of this study is ultimately to keep the consumer interest in the product as well as maintain its safety for consumption. (Badeka et al. 2003)

EVOH is a thermoplastic oxygen barrier used in many materials in the food industry today. It is used together with LDPE as a multi-layer composition coating inside Arla Foods yogurt cartons among others, and thanks to its barrier properties such as low oxygen permeability it will provide the anaerobic environment required to keep the yogurt fresh and edible. However, the material has a few drawbacks such as having a complex composition and a difficult manufacturing process which makes it an expensive material with few large-scale producers (Mokwena, Tang 2012). LDPE is a less expensive and simpler thermoplastic polymer made from petroleum and it is defined by its chemical and physical attributes. Thanks to its inertness at room temperature, low tensile strength and high resilience it is used in a wide range of products like plastic bottles, tubes, bags and wraps. This study will examine the impact a new coating will have on plain, strawberry- and vanilla-flavoured yogurt, and develop ways of potentially extending the solution to other dairy products.

1.2. Boundary conditions
The study was done at Arla Foods production facility located in Linköping, Sweden. A majority of the experiments were performed internally at site. The main part of the statistical testing was carried out at site, the rest were done at Arlas innovation centre in Stockholm. Physicochemical analyses and sensory trials were also done at site in the laboratory, using provided equipment and in collaboration with the workers. The chemical analysis in were outsourced and sent externally to the Technical Research Institute of Sweden in Gothenburg that had the certain expertise and instruments required. The yogurt used in this study was produced at Arla Foods Linköping, and packaged in the Gable Top 1 packaging-machine located in the company’s fruit section. Packaging cartons was provided by Elopak in boxes of 230 pcs. The study was designed to investigate mainly the difference between EVOH- and LDPE-based coatings in yogurt cartons and is a follow-up to Refthammars study where the same type of packaging was tested on cartons for extend shelf-life milk. This naturally leads to that some information in this study is based on previous work and now further investigated.

1.3. Expected impact of the study
This is an analytical study and was designed to investigate the impact of new packaging material and how it affects the contained food during a long-term storage. The outcome is based upon how well the new solution is perceived by the consumers since they are determining the demand of the products. It is also based on what the new solution can bring to the company in terms of effectiveness and profitability. A successful transition from EVOH- to LDPE-based coating will greatly benefit the company economically and give the possibility to expand and implement the same solution to other products. By changing packaging material for yogurt the company will be able to make savings in millions of SEK. It is estimated that the company puts around 200 million SEK yearly only on packaging material. The new material will also benefit in being easier to handle and store. Since the new packaging material is composed of a light low-density plastic coating, the handling and storing will be easier than with the traditional material. A low-density plastic made of polyethylene is also more recyclable since mono-materials of high purity are required in reprocessing (Bugnicourt et al. 2013).
1.4. Objectives of the work

The main objective of this project is to reach a conclusion about whether or not it is possible to switch the existing EVOH-based coating with LDPE-based coating inside plain and flavoured yogurt cartons without bringing about an unacceptable change to the yogurt. The yogurt made at Arla Foods dairy is designed to have a shelf-life of 28 days and during that time the product has to keep its appeal and quality to the consumer. During its storage time of 28 days the yogurt would be analysed and tested in order to detect changes in quality or organoleptic properties.

1.4.1. Guidelines and regulations

Food safety is one of the main concerns of the food and safety policy in the European Union. The food safety policies are constructed to protect consumer health and interests without a major impact of the market operations. The EU has a set of control standards to guarantee food hygiene, animal welfare and plant health to prevent the risk of contamination. It is important that new packaging material is in compliance with EU regulations. The policy establishes regulations on concepts such as traceability throughout the entire food production chain, including packaging. The framework regulation of (EC) No 1935/2004 establishes specific requirements on materials and articles intended to be in contact with food, it includes plastic materials and articles that are printed or/and covered by a coating. It contains definitions, restrictions and requirements such as:

Table 1.

<table>
<thead>
<tr>
<th>Article 3</th>
<th>Materials and articles, including active and intelligent, shall be manufactured in compliance with good manufacturing practice (GMP) so that they do not transfer any constituents that could:</th>
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<tr>
<td></td>
<td>• Endanger human health;</td>
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<tr>
<td></td>
<td>or</td>
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<td></td>
<td>• Bring about an unacceptable change in the food composition;</td>
</tr>
<tr>
<td></td>
<td>or</td>
</tr>
<tr>
<td></td>
<td>• Bring about deterioration in the organoleptic characteristics.</td>
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| Article 5 | Specific measures for groups of materials and articles may be adopted in order to ensure food safety such as: specific and/or overall migration limits (SML/OML). |
| Article 6 | Member states may maintain or adopt national provisions                                           |
| Article 16 | Declaration of compliance towards customer Supporting documentation: demonstrating compliance available on demand to the authorities. |

Since 2011, the EU enforced a new directive, the “Plastic implementation measure” as the regulation (EU) No. 10/2011. This is a specific measure within the 1935/2004 and establishes the following:

- A complete list of monomers, additives and other starting substances that can be used in plastic production for materials with food contact.
- Requirements (SML, OML etc.) that the material must fulfil when in use with the intended food simulant in real world conditions.
Materials for plastic packages, such as those used by Arla Foods should be in compliance with relevant food contact legislations. One of the objectives of this work is to make sure that the material intended to be used follows above standards by communicating with manufacturers.

1.4.2. Laboratory and analytical work
The analytical work is a tool to establish hygiene and quality in foodstuffs. The analyses in this study consist of four parameters that aim to investigate following attributes:

- Physicochemical
- Chemical
- Microbiological
- Sensory

Yogurt is a highly viscous, low-pH liquid made by bacterial fermentation of milk. The main objective is to investigate how the yogurt, based on the above four parameters, will be affected by changing the packaging board.

Physical properties are usually the most apparent flaws and can have the largest impact on the consumer. Yogurt with a defective appearance is likely to be thrown away at first sight, leaving other qualities such as taste and consistency totally irrelevant to the product. In order to continue with the new board the yogurt must at least have similar quality and durability as the original product during the 28 day period. The microbiological content is in this study analysed parallel to the physicochemical analyses since it can be considered as a part of the physical appearance.

A chemical analysis was designed to investigate if there is any difference between the reference and the test subject in their chemical composition. Volatile organic compounds usually constitute what the smell and the taste of the product is like. Through a chemical analysis it is possible to compare the levels of organic volatiles before and after the packaging switch, and also determine whether these components occur by chemical reaction, by migration from the packaging board or by other unknown means. As sub-objective this analysis was also performed to see if addition of flavourings has any impact on the yogurt’s properties e.g. masking potential off-flavours.

The final objective is measuring the organoleptic properties of the product. Both packaging materials are evaluated by both experts and regular consumers in sensory descriptive and discriminative trials of the yogurt. A trained expert is good at finding flaws in most food and is well suited for the task to give a descriptive feeling of the product through rating and comments. Consumers typically do not have the same spectrum of senses as the experts, but have the benefit of numbers.
2. Theory and methodology

2.1. Scientific background

The main principle in this project is to investigate how changing the inner coating from a complex EVOH-based multi-layer to a simpler, single-layer LDPE-based coating will affect yogurt. The quality and sensory attributes of the yogurt must maintain a certain degree of acceptance over storage of 28 days. There is on-going research about new and improved packaging material with better coating but few samples are available for commercial use.

2.1.1. Yogurt production

Yogurt is a consumable product made from a fermentation process of milk by the lactic acid bacteria species *Lactobacillus delbrueckii subsp. Bulgaricus* and *Streptococcus thermophilus*. Lactic fermentation is a very old and well known principle. It comes from the discovery of letting raw milk being ‘spoiled’ by microbes and giving it a firmer texture and a sour taste, the Turks called it “yoğurt” and is the accepted terminology of the product today. The process has been well refined over the years and is one of the most well-known and accepted method in the dairy industries around the world. Yogurt is made in different ways, plain or added with substances such as fruit, aroma components, gelling agents, sugar etc. The two bacteria have a stimulating effect on each other (protocooperation) and should exist in near equal numbers (1:1 ratio) to develop satisfactory attributes. (Walstra 1999)

The fermentation of milk consists of different metabolic pathways but is ultimately the conversion of lactose to lactic acid along with metabolites such as acetaldehyde, diacetyl and polysaccharides (figure 1). Lactose is a disaccharide and the major carbohydrate and main source of energy for most of the bacteria growing in milk. The bacteria hydrolyse the lactose into galactose and glucose, the glucose in turn is converted to lactic acid either through homo- or heterofermentative action shown in equations 1.1 and 1.2. Other metabolic pathways such as citrate metabolism, pyruvate dehydrogenase pathway and pyruvate-formate lyase pathway can also be entered depending on circumstances.

\[
\text{Homofermentative: } \text{Lactose} + 4\text{H}_3\text{PO}_4 + 4 \text{ADP} \rightarrow 4 \text{lactic acid} + 4 \text{ATP} + 3\text{H}_2\text{O} \quad [1.1]
\]

\[
\text{Heterofermentative: } \text{Lactose} + 2\text{H}_3\text{PO}_4 + 2 \text{ADP} \rightarrow \]
\[
2 \text{lactic acid} + 2 \text{ethanol} + 2\text{CO}_2 + 2 \text{ATP} + \text{H}_2\text{O} \quad [1.2]
\]

The produced lactic acid gives the milk a high viscosity, firmness, and lowering of pH, transforming it into yogurt. A p\text{H} about 4.6 destabilises certain complexes with an isoelectric point of the same value, resulting in a precipitation and coagulation of casein which gives the yogurt its dense characteristics (Germani et al. 2014). The two bacteria species have a stimulating effect on each other and can metabolise lactose much faster than each one would do individually. The proteolytic effect of *S. thermophilus* creates an abundance of small peptides and amino acids that together with lowered oxygen tension and formic acid stimulates *L. bulgaricus*. On the other hand *S. thermophilus* is stimulated by free amino acids and peptides released from milk proteins by *L. bulgaricus* (Horiuchi & Sasaki, 2012).
There are usually two common methods used to produce yogurt, depending on the preferences and the desired properties of the yogurt (figure 2). *Set* *yogurt* is made from milk that is heated to improve properties of the yogurt such as removing dissolved oxygen, kill bacteriophages, eliminate inhibitory substances etc. The yogurt is then cooled to inoculation temperature best fit for the culture used; usually this temperature is between 20 – 30 °C for mesophilic and 42 – 45 °C for thermophilic bacteria. The milk is then inoculated with a starter culture, packed and incubated during storage which means that the fermentation is on-going after packaging. *Stirred* *yogurt* is almost always made from non-concentrated milk that is also heated and then cooled before it is inoculated with a starter, similar to the set yogurt. After inoculation the yogurt is incubated (5 – 6 h) to form a gel. It is then stirred to obtain a smooth and firm product which is packed and stored. The stirred yogurt is fermented before packaging and is the type of yogurt that Arla Foods manufactures. (Bylund 1995a)
It has become very popular to add flavours and additives to yogurt in order to improve the sensations. Some common additives are fruit and berries processed as syrup or in a gel containing about 50% sugar and 15% fruit. The fruit mixture is added to the yogurt right before packaging, put at the bottom of the packaging or packed separately (Bylund 1995b). In this study plain, strawberry and vanilla-flavoured yogurt was used. Both strawberry and vanilla yogurt is manufactured by addition of fruit mixture before packaging.

2.1.2. Yogurt quality and oxidation
Yeast and mould growth is the most common cause of spoilage in plain and flavoured fruit-yogurt. It has the ability to grow in refrigerating temperatures and consume lactose and sucrose. Flavoured yogurt is likely to facilitate even more growth due to its high fructose and glucose content compared to plain yogurt (Mataragas et al. 2011). It is also known that oxygen has a considerable negative impact on yogurt and that continuous exposure to oxygen shortens the shelf-life through lipolysis. Although *S. thermophilus* and *L. bulgaricus* are aerotolerant anaerobes they only use most of the dissolved oxygen in the initial stages of fermentation. A study made by Marty-Teyssset et al. found that cultures of aerated and still (anaerobic) *L. bulgaricus* showed little difference in lactose consumption. The aerated...
culture had lowered the production of lactate because of an earlier entry into the stationary phase than
the still culture. However, the higher pH in the aerated culture indicated that the halt in growth was not
caused by acidic stress. It was suggested that the physiological change in the aerated culture was
related to oxidative stress and the production of superoxide radicals (O_2^-), hydrogen peroxide (H_2O_2)
and other radicals. (Marty-Teysset, De La Torre & Garel 2000)

Deterioration due to oxidation is a well-known problem in the food industry and of great concern for
all food manufacturers. Lipid oxidation can be caused in all sorts of food with 0.5 % or even lower fat
content and the off-flavours are organically detectable down to ppb levels which mean that even a very
small amount of lipid oxidation is capable of producing off-flavours. Oxidation in milk through
autoxidation is recognised as one of the major causes for shortening of shelf-life. Even though milk
differs from yogurt in many ways the mechanism of lipid oxidation is a three-stage reaction occurring
in the same way. Basically it consists of an initiation stage (equation 2.1) where a radical react with
the fatty acid hydrogen and forming an alkyl radical. In the propagation stage (equations 2.2 and 2.3),
the alkyl radical reacts with oxygen and forming peroxyl radicals which in turn react with other lipids
forming new alkyl radicals. The reaction chain is terminated when radicals react with each other
forming stable compounds, such as aldehydes, ketones, alcohols, alkanes and alkenes (equations 2.4
- 2.6).

\[
\text{Initiation: } RH \rightarrow R^+ + H^+ \quad [2.1]
\]
\[
\text{Propagation: } R^+ + O_2 \rightarrow ROO^-
\]
\[
ROO^- + RH \rightarrow ROOH + R^+ \quad [2.3]
\]
\[
\text{Termination: } 2ROO^- \rightarrow \text{non-radical products} \quad [2.4]
\]
\[
ROO^- + R^+ \rightarrow \text{non-radical products} \quad [2.5]
\]
\[
2R^+ \rightarrow \text{non-radical products} \quad [2.6]
\]

It is also possible for hydroperoxides to form by mechanisms in the presence of light (photo-
oxidation), where photosensitizers such as chlorophyll, riboflavin are activated by absorption of light.
The activated species can react by transferring electrons to lipids to form radicals that react with
oxygen similar to the autoxidation. The other way is that the photosensitizers are excited to form
singlet oxygen which can directly react with unsaturated lipids.

Lipid oxidation is a process dependant on various factors, many of them may be involved
simultaneously. Generally the factors involved are: concentration of oxygen, temperature, light and
innate properties of the medium such as lipid composition, amount of unsaturated fatty acids,
antioxidants, and also the physical appearance of the food. Lipid oxidation is normally not a big
problem in yogurt due to its low pH, low storage temperature and opacity (Serra et al.). One of the
important factors that influence the rate of oxidation is the amount of free lipids in the food matrix.
Once a gelated structure is formed in the yogurt, the fat droplets are embedded in the protein network
and isolated from the matrix, providing protection against oxidising agents (Velasco, Dobarganes &
Márquez-Ruiz 2010).

\subsection{2.1.3. Low-Density Polyethylene}
The food industry is continuously developing new food packaging technologies in response to
increasing customer demand of fresh, preserved and tasty products. The main focus in food
preservability lies on creating efficient barrier materials in regards to product safety and shelf-life
extension. Good packaging material prevents unusual flavours, light, microorganisms, oxygen and
other harmful substances to reach the food that could react with it and decrease its organoleptic properties, safety or quality. LDPE is a low-density polymer made of polyethylene which has high strength, light weight, high stability and resistance to moisture and is commonly used in food packaging material (Sirocic et al. 2014).

2.1.4. Ethylene-Vinyl Alcohol
Today Arla uses a multi-layer EVOH and LDPE solution to act as a barrier in many dairy products that need protection from the outside. EVOH is known for its excellent impermeability to oxygen, aromatics and oils, with the main drawback of being sensitive to moisture which affects its ability to work as an oxygen barrier (Huang et al. 2003). EVOH has become more and more common in food packaging thanks to its barrier properties and have been used together with LDPE to form a multi-layered structure with a broad range of properties (Mokwena, Tang 2012).

2.1.5. Comparison between LDPE and EVOH
All plastic materials used in packaging have a different degree of permeability to small particles such as oxygen, water and different aromatic compounds. To understand the behaviour between different barrier materials and how they react to external factors is essential when designing a good packaging solution (Siracusa 2012). Table 2 illustrates differences in properties between EVOH and LDPE as a barrier material.

<table>
<thead>
<tr>
<th></th>
<th>LDPE</th>
<th>EVOH</th>
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<tbody>
<tr>
<td>Oxygen barrier</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Max temperature (°C)</td>
<td>66</td>
<td>180</td>
</tr>
<tr>
<td>Tensile strength</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Cost</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Light barrier</td>
<td>Good</td>
<td>Poor</td>
</tr>
</tbody>
</table>

Since EVOH is sensitive to humidity it is used together with LDPE in a multi-layer solution to prevent absorption of water and ruining its barrier properties. This means that both types have the same material in contact with the food, which is LDPE. EVOH is known mostly for being an excellent barrier to oxygen. The oxygen transmission rates in formed cartons of EVOH in air have been measured to 0.2 – 0.5 ml oxygen/(package x 24h) while the reported OTR in LDPE is >100 ml oxygen/(package x 24h). It is therefore natural to think that products inside LDPE-boards are more exposed to oxygen and oxidative side effects.

Another aspect considering oxidation in foodstuff is the light penetration through the packaging and its dispersion in the food. As mentioned above, light is an important factor in oxidation effects on food, and the more exposure to light the greater the risk of photo-oxidation and deteriorated food. An EVOH-based carton has bleached fibres while the LDPE-based carton has unbleached fibres which makes the latter significantly better as a protection against light exposure.
2.2. Methodology

2.2.1. Evaluating sensory properties

When a food company decide to change their process of some sort it is necessary to investigate if there is any difference in the original product before the change to the product after the change. A common approach is to evaluate the sensory characteristics of the products, usually by experts trained for the task. Combining the expert opinions with a statistical difference test using a group of consumers, it is possible to establish any changes in the products organoleptic properties.

Sensory evaluations by a taste panel are the initial approach when comparing new and old dairy products. These evaluations can be described as a qualification process where the panel is tasked to determine any sensory off-characteristics in the new product. Panellists score the overall sensory characteristics on a scale, based on how well the product matches a specific quality standard. The experts provide an in-depth description of the product and justify their score to the absence/presence of certain characteristics, including defects. (Perez Elortondo et al. 2007)

A test of differences is an analytical test that is designed to determine if there are any sensory differences between samples. The most common methods in the food industry are pairwise comparison, duo-trio-tests or triangle-tests and they all constitute an answer to the question: “is there any difference between the samples?” and the answer is either “yes” or “no”. In statistical testing there are usually two types of errors that can occur and lead to wrong conclusions. Type I errors occurs when the null hypothesis is rejected when it is true, a false positive. Error of type II appears when no differences are spotted in a test that has differences. It is costly for companies like Arla to make errors of type II because any product changes that you thought were undetectable to the customer, that turned out to be detectable will affect the company brand negatively. The method chosen in this study is “triangle test” to determine any differences between yogurt products.

The triangle test is a discriminative method measuring if an overall difference is present over two test subjects. Three samples, two alike and one unique, identified by a randomly generated three-digit number, are presented to a group of individuals (figure 3). Each participant will taste, smell, feel and see all three products and tell if there are any differences. The more participants the more statistically significant the test will be since the amount of outcomes that occur by chance are reduced. After the test all the forms are corrected to see how many participants chose the right sample. The amount of correct answers are summed and compared with the amount of correct answers that would occur if the participants randomly made their choice. The significance of the result is determined by comparing the amount of correct answers to the tabulated value for triangle test. (Lundgren, 1981)
2.2.2. Sampling and analysis of volatile compounds in GC-MS/FID
The coating material inside yogurt cartons is the barrier between the product and the external environment and its main purpose is to keep the product intact and protected from harmful substances. It should also be designed to contain the product without bringing any contamination from itself. The quality of the yogurt will be examined by measuring the chemical composition at different points of time at certain temperatures, and then compare it with a reference. The results will be analysed and provide information about any substances that might have potentially brought about changes in any of the yogurts organoleptic properties. It also serves as to detect any foreign substances that make the yogurt inedible or even harmful to consume. Gas Chromatography (GC) is the most common technique employed when analysing volatiles in yogurt. Together with GC, the compounds in the yogurt can be identified in a mass spectrometer (MS), and quantified in a flame ionization detector (FID). GC-MS is probably the most popular method used in analysis of aroma in food thanks to its fast and accurate detection of organic volatile compounds. It has the capability to identify unknown compounds and describe their chemical nature. It can also detect, depending on the analyte used, levels down to picogram and therefore making it a highly sensitive instrument (Cheng 2010). By splitting the sample from the GC between a MS and a FID the capacity of the instrument is greatly enhanced. Using both detectors it is possible to provide a more detailed description of the compounds. Since volatile flavours are known to be primarily responsible for off-flavours in yogurt, this technique suits the purpose of this project well (Carrillo-Carrión, Cárdenas & Valcárcel, 2007).

In this study it was desirable to have a quick, relevant and reproducible sampling technique in order to fractionate the samples for the GC-MS/FID analysis. Dynamic headspace (DH) has been proven to offer a reliable, fast and reproducible method for sampling volatile compounds (Muresan et al. 2000), (Kim, Morr 1996). Because of the low intensity of the yogurt aroma, the samples are usually heated to extract volatiles of the compounds. Since the composition of sensitive aromas in yogurt are easily altered by heating, DH using a purge and trap at lower temperatures has been shown to offer a mild sampling technique (Ott, Fay & Chaintreau, 1997).

2.2.3. Physicochemical properties
All food companies want their products to last as long as possible without any spoilage and rejection from the customer. Concerning dairy products, the main reason for spoilage is due to growth of microorganisms that uses different kinds of substances in the product as an energy source. Stored yogurt also gradually builds a layer of whey, a harmless nutritious liquid with an unappealing look. To reduce bacterial growth inside dairy products they are packaged and contained in cartons which are treated with UV-light and hydrogen peroxide. One of the main focuses about changing the inner-
coating in yogurt cartons is to at least have the same shelf-life in the newly contained product as the original. (Vargas et al. 2008)

The physicochemical properties in this study are focused to the viscosity, whey content (syneresis), pH and microbiological growth in the yogurt. These properties are included as the most important characteristics and heavily related to customer acceptance. To examine the physicochemical nature of stored yogurt in two different cartons, several samples are picked and their properties are measured in a laboratory using appropriate equipment.
3. Materials and methods

3.1. Packaging material

The packaging material used in this study is 1 L gable top model branded Pure-Pak® Diamond delivered by Elopak on a special request. Empty and unfolded cartons called blanks are manufactured by printing, cutting and sealing of coated carton boards in a co-extrusion process. Co-extrusion is a process where two or more polymers are joined together and forming a continuous multi-layered profile where each layer has its own set of properties, making this kind of material more diverse and useful than a single layer material (Huang et al. 2003).

![Figure 4. Cut-through illustration of LDPE-based packaging material.](image)

![Figure 5. Cut-through illustration of EVOH-based packaging material.](image)

Two different types of boards used in this study are illustrated in figure 4 and figure 5. The LDPE-based carton is made of three layers of standard LDPE coating on the inside and outside with a paperboard in between. A majority of dairy products on the market are contained in LDPE coated containers since it is cheap and functional for products intended for short-term storage, 7-9 days (figure 4). For products intended for long-term storage (28 days), an EVOH-coated board is used for packaging. The EVOH-board is a complex material consisting of six layers of different coatings (figure 5). Besides laminating the inside and outside with LDPE, a layer of EVOH is attached between the inner LDPE and the paperboard with a special adhesive tie.

3.2. Packaging and storage

This study is based on three variants of private label yogurt produced at the Arla Foods factory in Linköping; 3.0 % plain, 2.7 % strawberry and 2.6 % vanilla. All three variants used the same packaging material and were treated equally (lighting, temperature etc.) during the tests. A total of 230 blanks were delivered, 26 blanks were reserved for machine calibration and leakage testing and the remaining 204 boards were divided into three different sized portions and reserved to each flavour: 82 L plain yogurt, 78 L strawberry cartons and 44 L vanilla.

Before filling the cartons with any product they had to fulfil certain standards set on packaging materials by Arla Foods. Since LDPE is more sensitive to heat than EVOH the packaging machine GT-1, had to be recalibrated to be able to seal the board properly. The temperatures for bottom and top sealing was lowered manually by 50 degrees, from 430 °C to 380 °C and 410 °C to 360 °C respectively. The sealing temperature has a major impact on the carton and an improper sealing will increase the risk of leakage. To confirm a proper sealing the cartons were tested with a blue ethanol-based liquid to see if there were any leakages. After passing the sealing and leakage tests the cartons were ready to be used in the production. Extra yogurt base, about 400 L, was added to the regular
production and the cartons were filled with product from the same batch. After the run the packages were transported to a cold storage in the laboratory to be stored for 28 days.

Analytical, chemical, sensory and statistical tests were performed at three different occasions during the 28 days of storage time. Since yogurt is a continuously changing dairy product there were three occasions; 5 days (start), 14 days (half-time) and 28 days (best-before) after production that were dedicated to testing (figure 6).

![Figure 6](image)
Figure 6. 
Timeline of the storage period and the planned analyses at 5, 14 and 28 days.

Measurements of whey-content, pH and stability were done after five days to obtain baseline data that shows the yogurts physical properties at the start. These analyses were coupled with a sensory evaluation to investigate the sensory attributes in the yogurt. Half way into the storage period the same measurements were performed as a follow-up to the first occasion and to see the gradual change in the product over time. The last tests were done after 28 days of storage which is the specified shelf-life of the produced yogurt. At this point measurements of both physical and sensory properties were done just as the previous occasions. Furthermore the yogurts chemical composition i.e. aromatic/volatile compounds, were analysed in a GC-MS/FID. Lastly a discriminative test was done by consumers and employees in order to detect potential differences between the test and the reference yogurt.

### 3.2.1. Sensory Evaluation

Three occasions during the storage period were assigned for sensory evaluation to detect any changes in the yogurt. The tests were descriptive and performed by a small group of trained experts at Arla. The location was a designated test room designed for sensory evaluation with the correct lighting, workspace, sound level, and ventilation. Panellists were presented with two pairs of three digit coded samples for each flavour (strawberry and plain), one reference and one test subject (figure 7). The following task was instructed to each panellist to examine and rate the appearance, consistency, smell and taste of the samples. The grades ranged from 1-9 where 1-3 points describe a product that deviates much from the product description, 4-5 deviates moderately and 6-7 describes small changes. A sample with 8 points is considered to be normal and 9 points is a product that exceeds any sensory expectations. Together with the rating the panellist was tasked to identify and describe potential off-flavours, odours or other deviations compared to a description of how the specified yogurt is meant to appear, taste, and smell. The score was written down on a test protocol (appendix I).
3.2.2. Statistical test
Following this sensory evaluation a triangle test was performed at 28 days of storage. A group of participants were introduced with nine samples split up between the three flavours in small white plastic bowls coded with randomly generated three-digit codes. Each test person was instructed to observe, feel (with spoon and mouth), smell, and taste all the samples in order to discriminate the sample that was different from the two others, how strongly it deviates and then mark it on the provided form (appendix II). Ten forms were prepared with different combinations of codes to achieve better statistical significance. The forms were corrected and compared to a tabulated value in order to determine if there is a significant difference between the products ($p < 0.01$).

To increase the statistical power each flavour received four codes that were randomly generated through the “RANDBETWEEN” function in excel. Ten forms were then prepared by randomly selecting three of the four digits, and place those in a randomised order on each form, also by using excel functions. The assigned numbers for each sort is shown in table 3.

Table 3.
Randomly generated numbers assigned to each flavour and packaging carton.

<table>
<thead>
<tr>
<th>Test subject (LDPE)</th>
<th>Test subject (LDPE)</th>
<th>Reference (EVOH)</th>
<th>Reference (EVOH)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain</td>
<td>369</td>
<td>271</td>
<td>781</td>
</tr>
<tr>
<td>Strawberry</td>
<td>814</td>
<td>658</td>
<td>465</td>
</tr>
<tr>
<td>Vanilla</td>
<td>407</td>
<td>626</td>
<td>935</td>
</tr>
</tbody>
</table>

3.2.3. Physicochemical analyses
All of the physicochemical analyses were done on plain and strawberry-flavoured yogurt. The pH of yogurt samples was measured 5, 14 and 28 days of storage respectively with a pH-meter. Two replicates were measured at day 5, and three replicates were measured days 14 and 28 for the plain and strawberry-flavoured yogurt. Syneresis was measured at 5, 14 and 28 days after production. By cutting of the top of the container with a knife, the yogurt’s surface was exposed where the liquid phase accumulates during storage. Any observed liquid was removed with a syringe and the volume measured in millilitres. For the viscosity analysis two yogurt samples for each flavour were stirred, put into plastic jars and stored in refrigerator for 24 hours. The viscosity of the yogurt was measured using a Brookfield DV-II viscometer with a size 4 spindle, 10 rpm and 1 minute running time.

Five samples from each flavour were prepared on a growth medium and put into incubation for five days at 24 °C. The samples were examined ocularly for any colony forming units (CFU) from yeast or mould and measured in CFU/ml.

3.2.4. Chemical analyses
A total of 12 samples were sent in a cool box to SP Food and Bioscience for analysis of volatile compounds in the reference and test packaging. The samples were stored in refrigerator at +5 °C until analysis. The sample preparation was performed by DH. 150 g of yogurt was put into Erlenmeyer flasks with long necks and special headspace-adapters. The samples were stirred by a magnetic mixer, Tenax TA adsorbent tubes were connected to the headspace-adapter inside the flask and the samples were conditioned in a water bath 25 °C for 30 minutes. The sampling started by leading helium into the flasks with a flow of 40 ml/min, when travelling out from the flask the helium carried the volatiles with it which were adsorbed on the tube wall. 1 litre of headspace gas was collected on the tube wall and the procedure was replicated for each yogurt sort.
The instrumental analyses were performed firstly by thermal desorption of the volatiles by an Automated Thermal Desorber 400 from Perkin Elmer, the gases were injected into a coupled ThermoQuest TRACE™ GC equipped with a TRACE™ MS and a FID which are operated parallel. The volatile gases were desorbed from the adsorbent tubes at 250 °C for 10 minutes with helium (100 ml/min) and split between the detectors by a branch connection. Helium was also used as the mobile phase in the GC at 3 ml/min, carrying the gases through the instrument. Temperature profile was set to a static 30 °C for 2 min, continuous increase by 4 °C/min up to 240 °C where it stayed for 5 min. A DB WAX etr 30m, 0.32 mm diameter, 1 µm thin film from Agilent Technologies was used as colon and considered as best suited for this analysis because of its high polarity and ability to analyse alcohols, solvents, benzenes, styrenes and organic acids.

By matching the peaks in the mass spectra to a reference value found in a library (NIST 2008) the identification of the unknown compound could be established. The comparison between the different compounds and their quantification were based on the chromatograms obtained in the FID. The quantification was performed through an external calibration where a known amount of hexanal was injected in the same way as the samples. The concentrations of the different components in the samples could then be observed as hexanal equivalents.

Besides analysing chemical and biochemical components, analysis of gas levels was also performed to study the fraction (in percentage) of gases (mainly O₂ and CO₂) in the headspaces. Single samples were taken from the headspace of the unopened yogurt carton by a syringe and analysed in a PBI Dansensor CheckMate II headspace gas analyser. The analysed gas levels in the headspace provide a representation of the fraction of these gases inside the yogurt bulk.
4. Results and discussion
The following section shows all results from physicochemical, chemical and sensory analysis at start, half-time, and best before date. There is also a section showing the results from the analyses at day 42.

4.1. Physicochemical and microbiological results
The following section shows the results from physicochemical measurements of pH, stability and viscosity at different occasions during storage. The microbiological results are also presented.

4.1.1. Physicochemical results

![Figure 8](image_url)

Figure 8.
The average pH at 5, 14 and 28 days for plain and strawberry-flavoured yogurt stored in LDPE- and EVOH-based cartons. The pH of vanilla-flavoured yogurt stored in LDPE and EVOH cartons are shown at day 28.

In figure 8 is the average pH value calculated from two samples at day 5, three samples from day 14 and five samples from day 28. The vanilla flavoured yogurt was only measured at day 28 due to an uncertainty that the amount of product would be enough for the remaining tests. During the storage period, the pH of all three sorts was held within the specified product limit of yogurt (≥4.0, ≤4.5).

As expected, the plain yogurt had a pH between 4.2-4.3 in the beginning while the strawberry-flavoured yogurt went a little lower. However the packaging board does not influence the pH of the product inside since the instrument has a margin of error of ±0.05.
Figure 9.
Measured average viscosity of plain, strawberry and vanilla yogurt packaged in LDPE- and EVOH-coated cartons at 5, 14 and 28 days of storage. Viscosity is measured in centipoise (mPa s).

The viscosity of plain and strawberry flavoured yogurt was measured at all three occasions, while vanilla was measured only at day 28. Both plain and strawberry had roughly the same viscosity over both packaging containers during the 5-14 day period. After 28 days the EVOH-packaged plain yogurt became less viscous than the plain yogurt in LDPE-coated packaging. Also noticeable is the higher viscosity in strawberry and vanilla compared to plain. This is usually explained by the fruit bits and pieces in the product, providing unevenness and a bit more resistance in the fluid. According to the product specification, fruit yogurt is recommended to have a viscosity between 5000 and 12000 cP. The strict limit is set at 5000 cP, any yogurt below 5000 cP is treated as a major defect and shall not be sold to customers. Any upper strict limit is not provided. Vanilla yogurt has a viscosity greater than 12000 cP, although outside the recommended interval it can still be delivered and sold to customers.

Table 4.
Stability measurements of five samples at day 28 representing each flavour and board. The numbers are given in percentages of the total amount of yogurt (1 L).

<table>
<thead>
<tr>
<th>Sample No. (%)</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDPE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EVOH</td>
<td>0.6</td>
<td>0</td>
<td>0.53</td>
<td>0</td>
<td>0.15</td>
</tr>
<tr>
<td>Strawberry</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDPE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EVOH</td>
<td>0.575</td>
<td>0</td>
<td>0</td>
<td>0.7</td>
<td>0</td>
</tr>
<tr>
<td>Vanilla</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDPE</td>
<td>0</td>
<td>0.325</td>
<td>0.3</td>
<td>0.35</td>
<td>0.725</td>
</tr>
<tr>
<td>EVOH</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

The ambition is to have a stability value of 0 %, preferably no more than 3 %. Stability value greater than 5 % is considered to be a “major defect”. While the analysed samples of yogurt stored in 5 and 14 days showed no signs of syneresis (0 %), the stability became slightly worse during the last days of
storage (table 4). The stability at day 28 varied between the samples, but none of them had any significant decrease in stability and is acceptable to deliver to market.

4.1.2. Microbiological results
To ensure safety and quality of the yogurt packaged in LDPE-coated cartons the microbiological growth was examined across all three flavours. There was no observable microbiological growth in any of the samples analysed through CFU counting on petri dishes.

4.1.3. Results day 42
The following section shows the results from physicochemical and microbiological analysis at day 42.

![Graphs showing viscosity, pH, and syneresis](image)

**Figure 10.** The results from the physicochemical and microbiological analyses on EVOH- and LDPE-packaged plain, strawberry and vanilla flavoured yogurt at day 42. All parameter values are calculated as an average from the measurements of three samples. The graphs show viscosity (a), pH (b) and syneresis (c).

There were no observed CFU on any of the petri dishes at day 42. Two samples of LDPE-packaged strawberry flavoured yogurt had a growth of mould on the inside of the screw caps. However, the
cause was established as a problem unrelated to the food but rather a fault in the packaging process and that the cap already was contaminated during the packaging, the yogurt itself was not affected.

4.2. Sensory evaluation results
The first evaluation at day 5 involved three experts who rated the yogurt and gave their opinion on its sensory attributes. The mean value of the participants ratings are shown, for the first trial in figure 11, second in figure 12 and the third in figure 13. Products with a score 6-7 are considered to have small weaknesses but can still be sold to customer, if the product receives a scoring of 5 or below it does not fulfil the requirements of yogurt products. A product given the score 4-5 has small to noticeable defects and not suitable for selling to customer. Scoring 1-3 is given to products with bad to extreme defects. That could be products with wrong colour, bad taste, containing foreign objects, and should not reach the market.

![Sensory scoring +5 days](image)

**Figure 11.**
The average scoring from seven panellists and their sensory evaluation of plain and strawberry yogurt stored for 5 days in LDPE- and EVOH-based packaging.

Five days after production, three experts gave their opinion on the sensory characteristics of the yogurt. The yogurt shows some weakness in smell and taste, especially the plain yogurt contained in the LDPE-based carton. All other properties are given a scoring at or above 7 which makes them perfectly acceptable for customer delivery. Some of the comments given to each sample are illustrated in table 5.
Table 5.
Comments and opinions from the sensory evaluation at day 5.

<table>
<thead>
<tr>
<th></th>
<th>Appearance</th>
<th>Consistency</th>
<th>Smell/taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain EVOH</td>
<td>• Few white grains</td>
<td>• Slightly thin</td>
<td>• Maybe some off-flavour</td>
</tr>
<tr>
<td>Plain LDPE</td>
<td>• Few white grains</td>
<td></td>
<td>• Oxidised taste</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• A small Off-flavour of “paperboard”</td>
</tr>
<tr>
<td>Strawberry EVOH</td>
<td>• Slightly weak</td>
<td></td>
<td>• Weakened aroma</td>
</tr>
<tr>
<td></td>
<td>colour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strawberry LDPE</td>
<td>• Few grains and</td>
<td>• Feels a bit</td>
<td>• Slightly weak taste</td>
</tr>
<tr>
<td></td>
<td>lumps</td>
<td>“fluffy”</td>
<td>• Some off-flavour</td>
</tr>
</tbody>
</table>

There are a couple of remarks on both flavours but mostly nothing serious except for the smell and taste of plain LDPE. A weakened aroma, colour or taste is generally nothing serious, but indications of alien off-flavours such as “paperboard” or “plastic” should be treated with more concern.

![Sensory scoring +14](image)

Figure 12.
The average scoring from seven panellists and their sensory evaluation of plain and strawberry yogurt stored for 14 days in LDPE- and EVOH-based packaging.

The result from the sensory evaluation at day 14 shows an overall better scoring than day 5. All of the samples are scored with a 7 or higher which indicates a perfectly acceptable product, but since the results are displayed as the average score from all participants it is not a perfectly accurate picture of their opinion. Some of the experts had some considerable remarks, specifically about smell and taste, pointing out “oxidative”, “cardboard” and “plastic” off-flavours. It is obvious that the results varied heavily from individual to individual.
Table 6. 
Expert comments from the sensory evaluation at 14 days.

<table>
<thead>
<tr>
<th></th>
<th>Appearance</th>
<th>Consistency</th>
<th>Smell/taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain EVOH</td>
<td></td>
<td>• Slightly “fluffy”</td>
<td>• A bit sour</td>
</tr>
<tr>
<td>Plain LDPE</td>
<td></td>
<td>• A bit rough</td>
<td>• “Paperboard” off-flavour</td>
</tr>
<tr>
<td>Strawberry EVOH</td>
<td>• A little thin</td>
<td></td>
<td>• Small oxidised taste</td>
</tr>
<tr>
<td>Strawberry LDPE</td>
<td>• Small syneresis</td>
<td></td>
<td>• Weak strawberry flavour</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Small bitterness/old flavour</td>
</tr>
</tbody>
</table>

It is apparent that the LDPE-based packaged plain yogurt contains traces of flavours that could be described as “oxidised” and “paperboard” (table 6). These results indicate that the LDPE-board does affect the plain yogurt and its organoleptic attributes, taste in particular. The LDPE-packaged strawberry flavoured yogurt was actually given a better score than the EVOH-packaged yogurt in terms of taste and smell, the expert comments indicates that a flavoured yogurt in this case is not particularly affected by the packaging board.

![Figure 13](image)

The average scoring from four panellists and their sensory evaluation of plain, strawberry and vanilla yogurt stored for 28 days in LDPE- and EVOH-based packaging.

The final sensory evaluation was done after 28 days of storage and also included the vanilla yogurt (figure 13). The scoring shows that the most affected attribute is still smell and taste while appearance and consistency seem to maintain acceptable levels. The largest difference in smell and taste between the boards can be observed in plain yogurt where the samples stored in LDPE-based cartons almost score a 5 on the scale, while its EVOH counterpart scores a 7.
Table 7.
Expert comments on the sensory scoring at day 28.

<table>
<thead>
<tr>
<th>Type</th>
<th>Appearance</th>
<th>Consistency</th>
<th>Smell/taste</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plain EVOH</td>
<td>• Few lumps and grains</td>
<td>• Thinner, rough</td>
<td>• Sour</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Taste of “old”</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Small bitterness</td>
</tr>
<tr>
<td>Plain LDPE</td>
<td>• Thick</td>
<td>• Slightly rough</td>
<td>• Oxidised</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Chemical/plastic aftertaste</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Slightly sour</td>
</tr>
<tr>
<td>Strawberry EVOH</td>
<td>• Thin</td>
<td>• Slightly rough</td>
<td>• Weak to no strawberry aroma and taste</td>
</tr>
<tr>
<td></td>
<td>• Small Syneresis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strawberry LDPE</td>
<td>• Small grains</td>
<td>• A little thin in mouth</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Weak strawberry taste</td>
</tr>
<tr>
<td>Vanilla EVOH</td>
<td>• Small grains</td>
<td>• A bit thin</td>
<td>• Weak vanilla aroma and taste</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>• Tastes a bit old</td>
</tr>
<tr>
<td>Vanilla LDPE</td>
<td>• Thick</td>
<td>• Slightly rough</td>
<td>• Sweet</td>
</tr>
<tr>
<td></td>
<td>• Small grains</td>
<td></td>
<td>• Slight loss of vanilla aroma and taste</td>
</tr>
</tbody>
</table>

The most common complaint was that the yogurt felt “rough”, had a few grains in it and had mostly lost its taste and aroma which was consistent across all flavours (table 7). The oxidised and plastic/cardboard flavour was still dominant in the plain yogurt contained in the LDPE-based cartons. It was also considered thicker and less sour than the EVOH variant. This attribute could also be observed in strawberry and vanilla, where the experts experienced a slightly thicker texture in yogurt contained in LDPE-based cartons. They also felt the fruit-flavoured yogurt in LDPE to be sweeter and less sour than the yogurt stored in EVOH. However, none of the samples were ever scored below 5 which means they could theoretically still be approved for delivery to the market.

4.3. Triangle test
Table 8.
Results from triangle test of customers, untrained employees and experts.

<table>
<thead>
<tr>
<th>Type</th>
<th>Participants (n)</th>
<th>No. of Correct answers</th>
<th>Minimum No. of correct answers $(p &lt; 0.01)^a$</th>
<th>Significance level (%) $^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Consumers</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain</td>
<td>47</td>
<td>25</td>
<td>24</td>
<td>0.4</td>
</tr>
<tr>
<td>Strawberry</td>
<td>48</td>
<td>24</td>
<td>25</td>
<td>1.2</td>
</tr>
<tr>
<td>Vanilla</td>
<td>48</td>
<td>27</td>
<td>25</td>
<td>0.1</td>
</tr>
<tr>
<td>Experts</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plain</td>
<td>12</td>
<td>7</td>
<td>9</td>
<td>6.6</td>
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<td>9</td>
<td>36.8</td>
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</table>

$^a$ J. Food Science 43(1978) 940-3.

The results from triangle testing are divided into a consumer group, where the participants are regular customers and untrained people from the factory, and an expert group where the participants are
trained experts from Arla Foods innovation centre in Stockholm (table 8). From the consumer test there were 25 correct answers out of 47 for the plain yogurt, 24 out of 48 for the strawberry and 27 out of 48 for the vanilla. The results indicate that the consumers could feel a difference between the samples in plain and vanilla yogurt \((p < 0.01)\). The significance derives from the fact that having equally or more than 25 correct answers for plain yogurt by pure chance is less than 0.4 \% and having equally or more than 27 correct answers for vanilla is less than 0.1 \%. The strawberry however did only receive 24 correct answers out of 48 and consequently a significance level of 1.2 \%. It is therefore statistically correct to assume that the consumers could not feel a difference between the samples of strawberry-flavoured yogurt \((p < 0.01)\).

Table 9.
Merged results of both testing groups from the triangle test.

<table>
<thead>
<tr>
<th>Type</th>
<th>Total Participants (n)</th>
<th>No of Correct answers</th>
<th>Minimum No. of correct answers ((p &lt; 0.01))</th>
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<tr>
<td>Plain</td>
<td>59</td>
<td>32</td>
<td>30</td>
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<td>33</td>
<td>30</td>
</tr>
<tr>
<td>Vanilla</td>
<td>60</td>
<td>32</td>
<td>30</td>
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</table>

Once the two test groups are summed up together the results are similar in the amount of correct answers (table 9). Since the number of correct answers across all three flavours just surpasses the minimum number of correct answers \((p < 0.01)\), it is clear that when merging the both testing groups results, there is a statistical difference between the yogurts. All participants were also tasked to leave a comment after each tasting and grade how strongly they felt the difference on a scale from “none” to “very strong”.

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The participants experienced an overall greater difference in the plain yogurt than in the strawberry and vanilla-flavoured yogurt. It is probably the result of plain yogurt being a sourer product with a little more bitter taste than the fruit-flavoured types, and any taste/smell differences are more prominent when there are no added flavours.

Figure 14. The graphs show the total number of people (experts and consumers) and their perceived intensity, from “none” to “very strong”, of differences between LDPE and EVOH stored (a) plain, (b) strawberry and (c) vanilla yogurt. A number of people market the intensity between two values which is displayed in the figures.
Even though all of the participants were asked to provide comments about their decision and their perceived sensation when tasting the yogurt samples, quite few actually did. The gathered comments are displayed in figure 15 and are categorised in specific sensory parameters like sourness, sweetness, thickness etc. There are obviously some mixed feelings in some sensations and while some people experienced a certain sensation in one product, other felt the opposite in the same product. But the consensus is that LDPE packaged plain and strawberry yogurt tasted a bit sweeter than EVOH which had a slightly more sour taste. The vanilla yogurt was more difficult to describe and the overall opinion from the participants is that the vanilla samples was very hard to distinguish. Even though there was a significant difference according to the triangle test, there is a small chance that the result occurred by chance (< 1 %).

**Figure 15.** The figures show the participants experienced sensation between the two different packaging materials during the triangle test of (a) plain, (b) strawberry and (c) vanilla yoghurt. The chosen sensations are picked from the participants own comments during the test, however most of the participants did not leave any comments at all, meaning that these results might not be entirely representative to all participants.
4.4. Chemical components

4.4.1. Gas levels
The result from the headspace gas analysis is presented in table 10 and shows the level of oxygen, carbon dioxide and nitrogen in the headspace of each sample compared to the surroundings.

Table 10.
Measured values in percentage from the gas analysis in the headspace of each yogurt sample.

<table>
<thead>
<tr>
<th>Board</th>
<th>Sample</th>
<th>O₂ (%)</th>
<th>CO₂ (%)</th>
<th>Rest (%)</th>
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<td></td>
<td>Room</td>
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<td>EVOH</td>
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<td>17,1</td>
<td>6,4</td>
<td>76,5</td>
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<td>18,2</td>
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<td></td>
<td>Vanilla</td>
<td>18,7</td>
<td>4,6</td>
<td>76,7</td>
</tr>
</tbody>
</table>

Although the results are from measurements of single samples, there is a clear difference in gas levels between the two boards. The gas levels in the LDPE-based containers are equalised to the same levels as the outside environment which is expected since low density plastics such as polyethylene are highly gas permeable and the systems are always striving for equilibrium. The EVOH-based containers on the other hand have a lower amount of oxygen and a 4 – 6 % amount of carbon dioxide. This is the result from having a container with lower gas permeability that effectively reduces the diffusion of gases through the carton. The presence of carbon dioxide could be explained in the lactic fermentation pathway where carbon dioxide is produced in by S. thermophilus during fermentation of milk which stimulates growth of L. bulgaricus (Angelov et al. 2009). Higher concentrations of carbon dioxide such as in EVOH is suggested to impact the flavour such as providing a more “sparkling taste” of the yogurt (Jansson et al. 2001).

4.4.2. Volatile compounds
The complete FID chromatogram and tables from the analyses of plain, strawberry and vanilla are shown in appendix IV. Comparisons between EVOH- and LDPE-packaged yogurt are illustrated in figure 16 where the concentrations of the components are shown in µg/m³.
Figure 16.
Detected volatile compounds in plain yogurt stored in EVOH- and LDPE-based cartons for 28 days. Concentrations are displayed logarithmically in µg/m³.

When comparing the components it becomes clear that there are certain but small differences between the EVOH- and LDPE-packaged plain yogurts. Visually, the components that differ the most in concentrations are the ketones diacetyl and 2,3-pentadione, and the alcohols ethanol, propanol and butanol which are all occurring in higher concentrations in the LDPE-based packaging, the compound styrene is also found at a higher concentration in the same packaging. The identified sulfides are all occurring in higher concentrations in EVOH-based packaging. The remaining components are considered to occur in the same or nearly the same concentrations in both samples in plain yogurt.
Figure 17.
Detected volatile components in strawberry-flavoured yogurt packaged and stored for 28 days in LDPE- and EVOH-cartons

The complete FID-chromatogram of the strawberry yogurt can be found in appendix IV-2. Same as the plain yogurt, the differences between the two packaging are small. The illustrated results (figure 12) shows the average value from three measurements and unlike plain and vanilla yogurt, the strawberry yogurt contain additional components. The biggest differences are still the diketones, diacetyl and 2,3-pentanedione, ethanol and butanol are also showing different concentrations as well as two of the sulphides. Propionic acid is another compound that is occurring in nearly the double amount in LDPE-samples than in EVOH-samples. The styrene is still visible in LDPE-samples, other than that the two packaging samples are pretty much the same. The esters visible in figure 14 are coming from the strawberry gel that is added to the yogurt base.
Figure 18. Detected volatiles in vanilla yogurt stored in EVOH- and LDPE-based packaging for 28 days.

When comparing the two subjects and the concentrations in vanilla-flavoured yogurt it is somewhat familiar with the two previous yogurt-types. The diketones (2,3-pentanedione and diacetyl) once again have a higher concentration in the LDPE-based packaging as well as the styrene. The alcohols; propanol and butanol are absent in the EVOH-based packaging and the sulphide-concentrations are higher in EVOH. The remaining components, including the vanilla specific vanillin are found in nearly equal concentrations in both samples. The complete MS/FID-spectrum for vanilla is shown in appendix IV-3.

When looking at the results from the chemical analysis it is difficult to notice any clear differences between the LDPE- and EVOH-packaged yogurts. The common attribute between the three flavours indicates a slightly higher amount of diketones (diacetyl and 2,3-pentadione) and alcohols (ethanol and butanol) in yogurt packaged in LDPE-boards. Alcohols and ketones are typical products that occur through lipid oxidation, where unsaturated milk fatty acids react with oxygen and forms odourless and tasteless intermediary products known as hydroperoxides. Hydroperoxides are unstable and will react with fatty acids and form carbonyl compounds (aldehydes, ketones) and alcohols, resulting in rancid off-flavours described as metallic, cardboard (Serra et al. 2008). Oxidation of foodstuffs is generally not a safety issue concerning human health but rather a matter of deterioration of the food quality in terms of degradation of its sensory characteristics.

4.5. Challenges and future research

To design and develop appropriate packaging material for yogurt, attention must be paid on certain characteristics such as pH, fat content, sensory properties and rheological attributes. This study shows promising results regarding the possibility of switching packaging material without affecting the product too much. According to the results from the sensory evaluation and the comments from the
participants, slight differences were suggested between the plain yogurt samples while it was more difficult to distinguish the different fruit flavoured samples simply by using the human senses. The greatest challenge remains, to develop a packaging material capable of protecting the food against external agents (oxygen) and migratory components while being economically viable. While the LDPE-coated board has the benefit of being a cheaper material, it suffers from being too permeable and lets oxygen penetrate and react with the food. Changes in the sensory characteristics of the food might be unnoticeable in instrumental analyses but the human senses are extremely sensitive (ppb) and cannot be fooled. While it is understandable that food companies are actively trying to cut costs through different activities, it is equally important that their products continue to be attractive to consumers. In a competitive environment, small changes that results in a slight deterioration of the product could have a devastating effect on the customer demand and number of goods sold.

Taking previous studies and this study into account, efforts should be put into highly viscous, high fat, low pH products that have a better resilience against oxidising effects. By adding fruit or similar additives (no aroma) to the product, it has a better chance to maintain a degree of acceptance to the consumer by simply overcoming and masking certain off-flavours. Another aspect that could be considered in the triangle test is to ask the participants which one they preferred instead of just identify the different sample.

Active packaging is an innovative concept in the matter of protecting food from lipid oxidation. Several active substances inheriting different antioxidant mechanisms have been studied as a part of an active packaging system, most predominantly the migratory packaging system. The non-migratory packaging system is beginning to receive more interest as customers become more aware of food additives. Both systems still require more research in order to become commercialised and economical viable. (Tian, Decker & Goddard, 2013)

4.6. Final thoughts

It is evident that this work has proven the theory that yogurt is a dairy product that is less susceptible to oxygen and oxidation compared to ESL milk. The study on ESL milk concluded confidently that there was a rather large difference between milk stored in LDPE-based packaging compared to those stored in EVOH-based packaging. This work shows that the same effect that occurred in the milk, also occurred but to a lesser extent in yogurt. Changing of packaging material has made the most impact in plain yogurt, probably because it is an un-flavoured product where certain off-flavours are more distinguishable than flavoured ones. The change in flavoured yogurts did not necessarily make it less attractive.

The question that remains is: “Are the economic benefits worth the potential risks for consumer disapproval?”. In a competitive environment, there is a fine balance between keeping the right product quality and cutting costs. Implementing a change should also be easy to integrate in the existing process to keep a continuous flow. Changing to a cheaper packaging material is not favourable if the change generates higher costs in other parts of the process that nullifies the benefits. The implementation should be considered in packaging lines where mostly yogurts are packaged. As long as Arla has the capability to adjust to a new material and integrate it with the existing process, a change of packaging material is recommended. The changes in the sensory characteristics are not necessarily making the yogurt worse, only different.
5. Conclusion
Based on the gathered results from the sensory evaluation and the triangle test the participants were able to detect differences between yogurt stored in LDPE-coated cartons and yogurt stored in EVOH-coated cartons across the three flavours. Although this result is statistically significant ($p < 0.01$), the personal experience and the opinions of the participants clearly shows that the differences are vague and hard to identify, especially in fruit flavoured yogurt, as was hypothesised before the study. The biggest difference that could be detected was found in plain yogurt. The plain yogurt was described to have a slight taste of “chemicals”, “cardboard” and “plastics”, which is the classical symptom of an oxidised product. Based on the physicochemical results, there are no significant differences between the yogurts in pH, viscosity, stability or microbiological shelf-life. The chemical analyses indicated differences, albeit small, in certain volatiles naturally occurring in yogurt and are likely to be connected to the sensory experiences to that of an oxidised product. The compound styrene is found in LDPE-packaged yogurt in small amounts. In summary:

- It is in fact not possible to change the packaging material on Arla Foods 3% plain, strawberry and vanilla yogurt without influencing its sensory characteristics.
- Results from sensory, statistical and chemical analyses indicate that there has been an occurrence of oxidation in the yogurt as a result from higher gas permeability of LDPE.
- The biggest differences in sensory attributes were observed in 3% plain yogurt.
- The physicochemical characteristics of yogurt were not affected by a change of packaging material.
6. Acknowledgments

The author acknowledges Arla Foods AB for the outstanding support and opportunity of providing a subject a master thesis could be based upon. Thanks to supervisors Robert Gustavsson and Mette Toft for excellent guidance throughout this work. Special thanks to Thomas Nilsson for contributing with in-depth knowledge of the dairy industry and products, Patrik Libander for the work of identifying chemical compounds and giving valuable input in this matter, Johan Bergholm for cooperation with Elopak, and Maja Bondesson and Gunilla Linde for their guidance in the laboratory.

Thanks also go out to colleagues Johanna Hoxell and Linda Liepina for their outstanding support during the triangle test, Hampus Tengelin for providing valuable input and comments during the study. In general, employees at Arla Foods AB have all been very supportive and forthcoming during the work period.
7. References


http://www.arla.com/Global/about/portlets/Brief%202014/Arla_Brief_ENG_web150.pdf


Cheng, H. 2010, "Volatile flavour compounds in yogurt: A review", *Critical reviews in food science and nutrition*, vol. 50, no. 10, pp. 938-950.

European Commission 2015. Food and Feed Safety. Legislative lists available at:
http://ec.europa.eu/food/food/chemicalsafety/foodcontact/legisl_list_en.htm


Appendix I

Testprotokoll

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Skriv felord efter felordslusten till alla poäng lägre än 8. Om felord saknas försök att beskriva felet.

Figure I-1.
The test protocol used for the descriptive sensory analyses.
Appendix II

**_TRIANGELTEST_

Produkt: Yoghurt

- Mild Naturell 3.0 % Fetthalt.
- Mild Vaniljyoghurt 2.6 % Fetthalt.
- Jordgubbsyoghurt 2.7 % Fetthalt.


**_NATURELL_

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*Kommentar:

**_JORDGUBB_

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**_VANILJ_

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*Kommentar:

Tack för din medverkan! Resultatet presenteras vid ett senare tillfälle.

**_Figure II-1._

The form used in triangle test
Appendix III

Figure III-1. The cold storage temperatures during the 28 day storage period.
Figure IV-1. FID chromatogram. Plain yogurt, LDPE packaging in red, EVOH packaging in black. Retention time 0-60min.
Figure IV-2. FID chromatogram. Strawberry yogurt, LDPE packaging in red, EVOH packaging in black. Retention time 0-60min.
Figure IV-3. FID chromatogram. Vanilla yogurt, LDPE packaging in red, EVOH packaging in black. Retention time 0-60min.
### Appendix V

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