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Investigation of active packaging methods to improve quality and shelf life of meat

Miriam Aljarroudi

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Abstract

It is well known that *Active packaging*, like the use of modified atmosphere, gives an improved quality on fresh food products and extended shelf life. The need of active packaging is partly due to the increasing demand of food that is minimally processed, is easily preserved, fresh and with a long shelf life. In the recent years, it has also been a greater focus on limiting the use of preservatives in food. These criteria present challenges for food safety and food quality, and driving a search for innovative ways to inhibit microbial growth in food, maintaining quality, freshness and safety. Antimicrobial packaging is a promising form of active food packaging, in particular for meat products.

The intention of this study was to investigate the effect of a specific antimicrobial film as packaging material in combination with modified atmosphere packaging (MAP). And, also compare this to the APET/PE packaging with MAP, on quality and shelf life of beef loin steak. The effect of the packaging methods, combination of gas atmosphere and packaging material, was evaluated by monitoring the gas content, color, drip loss, pH and microbial growth on beef loin steaks. Samples were examined after 9, 16, 22, 27 and 30 days of storage at 4 °C.

Slices of beef loin steaks were packed in two different materials, one antimicrobial and one consisting APET/PE. Two different packing methods were used for both materials; vacuum and modified atmosphere packaging, with two different gas atmospheres were used, 60% CO_2/ 40% N_2 and 75% O_2/ 25% CO_2.

The results show that the antimicrobial packaging had no better inhibition of bacterial growth compared to the modified atmosphere packaging. Packing methods however, had a greater effect on the inhibition of bacterial growth. The storage stability of the vacuum packed meat was relatively for 22-25 days, and MAP consisting of 60 % CO_2/ 40% N_2 about 30 days. When it comes to the storage capability of MAP, high oxygen consisting of 75 % O_2/ 25 % CO_2, it was also about 30 days.

But the bacterial growth was unexpected, extremely low. This seems strange considering that meat stored in high oxygen, usually turns bad long before meat packed in CO_2. The values for color, liquid loss, and the pH of the meat in the two package materials were nearly identical, but the a* value (redness) was higher, and the fluid loss was slightly lower for the meat in antimicrobial packaging.

The main conclusion is that modified atmosphere packaging with CO_2/N_2 in combination with APET/PE packaging had the best effect in increasing the quality and durability, considering that high oxygen eventually will result in a rancid flavor of the meat. While the antimicrobial packaging had no generating effect of inhibiting bacterial growth, but resulted in lower drip loss and higher a * - and b * values in relation to the meat with APET/PE packaging.

Sammendrag (Norwegian summary)

Det er kjent at aktiv emballering, som bruk av modifisert atmosfære, gir en forlenget holdbarhet og forbedret kvalitet på ferske råvarer. Behovet for aktiv emballering kommer delvis av en økende etterspørsel for mat som er minimalt bearbeidet, lett å tilberede, ”fersk”, og med lang holdbarhet. I de senere årene har det også vært et større fokus på å begrense bruken av konserveringsmidler. Disse kriteriene byr på utfordringer i forhold til matvaretrygghet og kvalitet, og dette driver en søken etter innovative måter å hemme mikrobiell vekst, opprettholde kvalitet, ferskhet og matvaresikkerhet. Antimikrobiell emballering er en lovende form for aktiv emballering av kjøttprodukter.

Målet med denne oppgaven var å undersøke effekten av en utvalgt antimikrobiellemballasje i forhold til modifisert atmosfære pakking (MAP) for å forbedre kvalitet og holdbarhet på storfekjøtt. Effekten av pakkemetodene, kombinasjon av gass atmosfære og pakkemateriale, ble evaluert ved å overvåke gassinnholdet, farge, væsketap, pH og mikrobiell vekst på ytrefilet fra storfe. Prøvene ble analysert etter 9, 16, 22, 27 og 30 dagers lagring ved 4 °C. Ytrefilet fra storfe ble pakket i to ulike materialer, en antimikrobiell film og en film bestående av APET/PE. To ulike pakkemetoder ble benyttet for begge materialene; vakuum og modifisert atmosfærepakking med to ulike gas sammensetninger (60% CO_2 / 40% N_2 and 75% O_2 / 25% CO_2).

Resultatene viser at den antimikrobielle emballasjen ikke hadde noen bedre hemming på bakterieveksten sammenlignet med modifisert atmosfærepakking. Pakkemetodene hadde derimot en større innvirkning på hemming av bakterieveksten. Lagringsevnen til vakuumpakket kjøttet var henholdsvis 22-25 dager, og for MAP; 60% CO_2/ 40% N_2 ca. 30 dager.

Når det kommer til lagringsevnen til MAP; høyoksygen (75 % O_2/ 25 % CO_2) , var den også utfra resultatene ca.30 dager. Men her var det uventet veldig lav bakterievekst, noe som virker rart med tanke på at kjøtt i oksygen holder dårligere enn kjøtt pakket i CO_2. Verdiene for farge, væsketap og pH på kjøttet med de to pakkematerialene var tilnærmet like, men a* verdien var noe høyere og væsketapet var noe lavere for kjøttet med antimikrobiellemballasje.

Hovedkonklusjonen er at modifisert atmosfære med CO_2/N_2 i kombinasjon med APET/PE-emballasje hadde best effekt for å øke kvalitet og holdbarhet, med tanke på at høyoksygen etter hvert vil resultere i at kjøttet får en harsk smak. Mens den antimikrobielle emballasjen ikke hadde noen frembringende effekt på å hemme bakterievekst, men resulterte i et noe lavere væsketap, og noe høyere a*- og b* verdier, i forhold til kjøttet pakket i APET/PE.

Contents

1. Introduction

What impact has active packaging, with the use of antimicrobial film, on meat product quality and shelf life? Can antimicrobial packaging combined with modified atmosphere- or vacuum packing improve the quality and give an extended shelf life of meat compared to the use of "regular" packaging material without any antimicrobial components? Does an antimicrobial film has any effect on the product and are there any differences in the results using different packaging methods?

The aim of the study was to examine if a silver ion-based film along with different atmosphere had a positive effect on shelf life and quality, compared with an APET/PE packaging. The type of meat that was used in the research project was sirloin from cattle that was pre-tenderized, because this type of meat has a tendency to look quite similar, homogenous; which ensure a minimum of variation between samples. It was used three different packaging methods with the two different types of packaging material. One material with antimicrobial agents, and one without, used as a standard sample to compare and show if the antimicrobial packaging material had any affect.

2. Aim of the study

The aim of this study was to investigate the effect of a specific antimicrobial film in packaging of beef loin steaks, regarding to the quality development and shelf life during cold storage. This overall goal was divided into the following tasks:

- To compare antimicrobial packaging with modified atmosphere packaging
- To compare antimicrobial packaging in combination with modified atmosphere versus modified atmosphere packaging
- Examine the effect of silver ions incorporated in the packaging material

In order to measure quality and shelf life of beef lion steaks, the research had a focus on gas composition, color, drip loss, pH and bacterial counts.

3. Theory

3.1 Food packaging

The primary package is the one that comes in direct contact with the food, and is therefore the most important component of the packaging process. In addition to the primary package there are commonly also secondary and tertiary packaging systems involved (Nollet et al. 2012).

Why use food packaging? A few years ago, you could only buy apples in loose weight, whereas now you can also buy apples in small cardboard tray with plastic film around. The primary purpose of food packaging is to protect the food during handling, storage, transport and sale of the product and to maintain good food quality. Packaging prevent damages, chemical changes and microbial contamination, gas, dust and odors (Nollet et al. 2012), (Sung et al. 2013).

The packaging material applied should be appropriate for the type of storage conditions the product is exposed to. And the type of packaging material used will determine the visual appearance, which is of extreme importance if the product is to be consumes as a fresh item (Nollet et al. 2012).

Packaging and packaging conditions must be adapted to the various requirements of food products. A good packaging should act as a barriers system to reduce passage of surrounding contaminants into foods (Sung et al. 2013). Most food products are sensitive to oxygen, light, temperature and microbial contamination. A combination of oxygen and light will give a discoloration of meat products. Fat products develop an oxidized taste and odor when they are exposed to light and air, or during prolonged storage at room temperature. Unpackaged food will become dry on the surface and the risk of microbial contamination of the product increases. In addition to providing the best possible protection of food products, the packaging must also be suitable for transport, storage and promotion. The packaging also has to provide details about contain, the manufacturer, weight, composition, durability, storage method and place of manufacture (Eie 2007).

In most fat containing products an oxygen barrier is needed to prevent oxidation and flavor errors, and also to prevent or reduce the destruction of vitamins and antioxidants. An aroma barrier is often linked to the O_2 barrier. Good aroma barrier prevents volatile aromas and perfumes to get through the packaging material. An aroma barrier will also prevent odor from other items affecting the taste of the product inside the packaging (Eie 2007).

Carbon dioxide barrier is necessary for packaging of "living" products, the CO_2- review have to be adapted to the product production of CO_2 and its needs for O_2. When perishable products are being packed, it is important that the added CO_2 gas does not escape. Water vapor penetration depends on the thickness and type of polymers. Exposure of light is devastating for most foods, especially in the presence of O_2 (Eie 2007).

3.2 Packaging materials

3.2.1 Polymer

The word Polymer is derived from Greek, were the root *meros* has the meaning parts, and *poly* meaning many. A polymer is a compound consisting of long-chain molecule, made up of repeating units, monomers (Pettersen 2004). The properties of polymers are determined by their molecular structure, molecular weight, degree of crystallinity and chemical composition. These factors in turn influence the density of the polymers, and the temperatures at which they undergoes a physical transition (Robertson 1993).

There are two main types of polymers

- Homopolymers: consisting of the same repeating unit
- Heteropolymers: consisting of two or more different units, regularly or irregularly. And are referred to as copolymer when two different monomers are polymerized together

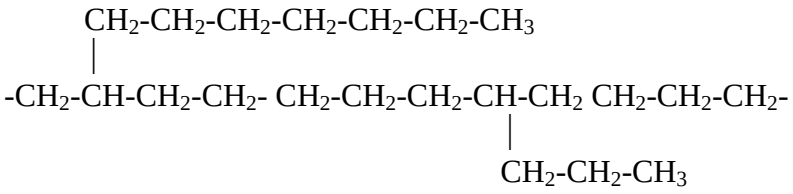
Polymers can also exist as oriented form. The orientation of polymers has the gain to improve their strength and durability in order to expand their ambit and make them serviceable in thinner devices. Orientation is a process of stretching the material, to line up the molecular chains in a predetermined direction. The film may be oriented in either one direction (uniaxial

orientation) or, in two directions, usually at right angels to each other (biaxial orientation) (Robertson 1993).

3.2.2 Polyethylene

Polyethylene (PE) or more specific a low density polyethylene, LDPE, one of the polyolefins of PE are used in both laminates in this research. PE is one of the simplest polymer, and the most common used in packaging materials. PE molecule is built up by the monomer ethylene (C_2H_4). Properties such as density and melt index are key characteristics regarding processing and use properties of the different polyolefins. LDPE are a soft thermoplastic with low density. (Robertson 1993)

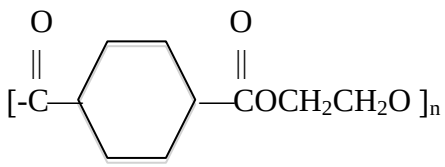
LDPE is a polymer consisting of long hydrocarbon chains, whit short and long branches with a terminal methyl group.



The occurrence of these branch chains prevents a close packaging of the main polymer chains. Areas where the chain is parallel and closely packed are largely crystalline while the disordered areas are amorphous. PE is a though polymer with slightly translucent material (Briston & Katan 1989).

3.2.3 Polyethylene terephthalate

Polyethylene terephthalate, PET also known as polyester, is a large group of polymers with ester linkages and a sequence of carbon- carbonyl- oxygen- carbon.



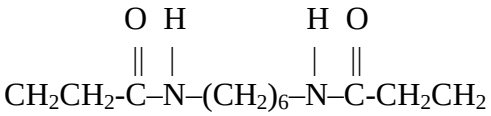
PET

APET polymer is prepared from monomer ethylene glycol and terephthalic acid. It contains the elements carbon, hydrogen and oxygen and a portion of the chain is aromatic. Without any further processing of PET it remains a predominantly amorphous structure known as polyethylene terephthalate, APET (Robertson 1993).

The polymer is very rough and strong which makes it resistant to abrasion. PET have also excellent transparency. The slip characteristics on the on hand are poor unless slip additives are incorporated. PE has low water vapor permeability, and their permeability to gases and odors is also fairly low (Briston & Katan 1989).

3.2.4 Polyamide

Polyamide (PA), has a sequence of carbon- carbonyl- nitrogen (amino)- carbon, and contains one amid group -CONH. Compered with polyester, the oxygen is switched with an amino group (Robertson 1993).



PA can be prepared by condensation of certain ω-amino acids. The number of carbon atoms in the parent amino acid determines the type of nylon. The ones that are most commonly used in film from are nylons 11, 12, 6 and 6.6. PA is a rough material with high tensile strength and good resistance to abrasion. PA has very good gas barriers, but also a high water absorption which can consequently affect their mechanical properties. The effect is not permanent and full properties can be restored on drying. (Briston & Katan 1989).

3.2.5 Ethyl vinyl alcohol

Ethyl vinyl alcohol, EVOH is a copolymer of ethylene and vinyl alcohol with varying content of ethylene. Which can be made by the hydrolysis of poly vinyl acetate. It has a high percentage of –OH groups, which makes EVOH hydroscopic (Eie 2007).

EVOH are highly crystalline, with properties that are very much dependent on the relative concentration of the comonomers. EVOH has a high mechanical strength, surface hardness

and elasticity. It is also highly resistant to abrasion and has excellent weatherability. But their outstanding characteristic is their ability to provide a barrier to gases such as oxygen, nitrogen and carbon dioxide. The extremely high gas and solvent barrier propertied- are reduced under humid conditions. But in dry conditions there are no other polymers that have such good barrier properties to oxygen. EVOH need a (polyamide or polyethylene) layer to protect it against moisture to maintain the good barrier properties. (Briston & Katan 1989)

3.2.6 Food packaging

A single polymer is not suitable for package in most application, as it is unable to provide all the necessary properties to create appropriate barrier characteristics for instance. Polyolefin; polyethylene (PE) and polypropylene (PP) are the most widely used polymers for food packaging purposes. They have excellent heat sealing and good humidity barrier properties, but are rarely used alone, because of their poor gas barrier properties. Polyvinyl chloride (PVC), polyamides (PA), polyethylene terephthalate (PET) and ethylene-vinyl alcohol (EVOH) are polymers that can provide good gas barrier properties, but are sensitive to humidity. Food packaging is usually multilayer constructions, incorporate with a type of resins. Laminates or coextruded films normally consist of two or more materials, and usually a center layer, which provides superior gas barrier properties and inner layer with heat-sealing properties are involved. And an outer layer that acts as a water barrier, or tie-layers (Pettersen 2004).

3.3 Thermoforming

Thermoforming machines produce packaging by a bottom film and a top film. The bottom film is thermoformed, shaped like a tray with room for the products to be packed. The tray form occurs after the film has been heated to approximately 100-120 °C, to make it soft and conformable. The process is called thermoforming. The top film and the bottom film are brought together into a closed vacuum chamber, the welding tool. In the welding tool, air is removed from the package, known as vacuum, or supplied a gas mixture. The welding plate press the top film and the bottom film together and seals them along the edge of the bowl. In the final step of thermoforming, the packages are separated with a knife (Eie 2007)

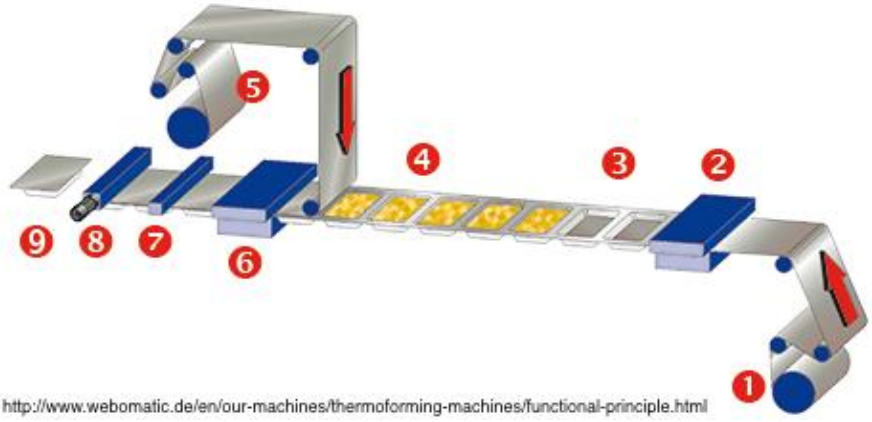


Figure 3.1 Functional principles for thermoforming. 1: Bottom film is unwound from the roll. 2: Forming station, where the bottom film is heated and formed. 3: The formed package~~s~~ → transported forward. 4: Filling area; where the package is filled with products. 5: Top film is unwound from the roll. 6: Sealing area; the package is put under vacuum and modified gases are added if necessary. The top film is then sealed to the bottom film by heat and pressure. 7 and 8: Cutting of the package. 9: Finished – ready for further transport.

3.4 Active packaging

Active packaging, insinuate changes. The packaging method has an effect on the surroundings of the packaged product, and the changes are made to give the product an extended shelf life, improve the health security and maintain good sensory characteristics. The need of active packaging is partly due to the increasing demand of food with a minimum of food additives and preservatives, that still looks fresh and tasty, and have long shelf life. A long shelf life is convenient, both for the consumer and the producer. Types of food storage have over time changed from canned and frozen food, to the desire for fresh food. These criteria present challenges for food safety and quality (Quintavalla & Vicini 2002).

The retail industry has also changed, which increases the need of active packaging. There has been a centralization of activities such as stock and production facilities.
More efficiency, and wastage reduction is needed to increase the profit. The distribution distances for food are extended. Therefore it is necessary to develop packaging concepts that gives food a long shelf life and ensure the food safety (Eie 2007).

Active packaging can be divided in absorbers and emitters. An absorber removes undesirable components in the atmosphere around the food product to extend shelf life and/or improve the

quality. This can be components such as oxygen, carbon dioxide, ethylene gas, humidity, undesirable odor or taste. An emitter on the other hand, adds components to the atmosphere around the food product to extend shelf life and/or improve the quality. This may be components as antioxidants, antimicrobial, carbon dioxide, aroma and lactase (Eie 2007)

3.5 Modified atmosphere packaging and vacuum packaging

Air is composed of 78% nitrogen (N_2), 21% oxygen (O_2), 0.03% carbon dioxide (CO_2) and 0.97% noble gases. O_2 has a negative effect on most food. Many spoilage bacteria are dependent on O_2 to live and reproduce them self. If pigments are exposed of oxidation, fat proteins leads to color changes, rancidity, odor problems and poor water holding capacity of proteins. Vacuum and modified atmosphere packaging are two of the most important methods for packaging of fresh meat, and are used in this research project (Eie 2007).

3.5.1 Modified atmosphere packaging

By using *modified atmosphere packaging* (MAP), the atmosphere around the food products is changed by supplying a desired gas mixture. The packages will not be controlled or adjusted during the storage period, but the atmosphere will change over time. The changes depend on the type of food and may be related to absorption of CO_2 in the product, and the gas barrier of the packaging material. There are many reasons for atmosphere changes during storage, such as microbiological activity of different bacteria and yeast produces CO_2 gas and consume O_2. The features of the packaging material contribute to the changes. O_2 may penetrate and CO_2 may escape depending on barrier properties, temperature and humidity. Poor sealing and damage occurred before, during or after packaging has a large and rapid impact on the gas composition inside the package. (Eie, 2007)

MAP is used for retail packaging of food products. A gas mixture replaces the air, wherein the amount of each gas is determined by the time of packaging. The reasons for the increased use of MAP are that the consumers want fresh, chilled products without preservatives. It also provides an extended durability and efficiency gains in the production and distribution of

fresh chilled products. Problems associated with vacuum packaging are; liquid excretion, and deformation of products are also avoided (Roberts & Skinner 1983).

Nitrogen, oxygen, carbon dioxide alone, or in combination, are the most common gases used in gas packaging. The main functions of nitrogen gas in gas packaging, is to displace oxygen and function as a fill gas to prevent package collapse. When oxygen is displaced, the growth of aerobic bacteria, mold and yeast, oxidation of fats and pigments and development of undesirable odors is reduced. N_2 do not have any direct bacteriostatic effect. It is used as a filling gas to avoid a vacuum effect by high concentration of carbon dioxide. N_2 has a low solubility, and because of that it does not dissolve in fat fraction as carbon dioxide does. (Eie, 2007)

Gas packaging will normally reduce the amount of oxygen in contact with food products. O_2 has an effect on color; oxidation of pigments causes color changes. Microorganisms like molds and aerobic spoilage bacteria such as *Pseudomonas*, requires O_2 to grow. Oxidative rancidity of fats occurs only when the food has access to O_2. In some types of food it is desirable to have a certain percentage of O_2 in the gas mixture. 1-2 % oxygen is sufficient to inhibit the growth of anaerobic microorganisms. When packaging fresh meat, a high percentage of O_2 sometimes is used (in the gas mixture) to stabilize the color of the meat. The fresh red meat pigment (substance), oxymyoglobin, requires 70-80% O_2 in the gas mixture to maintain the red color during the storage period. (Eie, 2007)

CO_2 has an antibacterial effect. A displacement of O_2 will reduce the opportunity for a growth of aerobic bacteria. CO_2 has the ability to readily penetrate bacterial cells, which will enhance the ability to inhibit cell metabolism. CO_2 also seems to acidifying the cells pH and may therefore reduce metabolic activities. (Eie, 2007)

CO_2 has varying effects on different microorganisms. Mold, some yeast species and gram-negative bacteria are effectively inhibited by CO_2. Gram Positive (as *lactic acid bacteria*) is only inhibited in a small degree. (*Lactic acid bacteria* are a small part of the natural micro flora of meat. A high proportion of *lactic acid bacteria* will not immediately give a stale and inedible product; they only become sour /acidic after several weeks of storage). By using CO_2 the micro flora are steered towards a more harmless spoilage flora. (Eie, 2007)

The inhibitory effect of CO_2 gas increases when the concentration is increased. An effect is achieved at concentrations above 20 %. The effect also increases when the temperature is decreasing, because decreased temperature increases the solubility of CO_2. A combination of CO_2 atmosphere and a storage temperature of 4 °C or lower will provide the best antibacterial effect. On the other hand high concentrations of CO_2 may also have a negative effect on the color and increase fluid secretion. (Eie, 2007)

CO_2 extends bacteriological shelf life on meat from 3 to 10-14 days compared to storage exposed to air. N_2 serves as the filling gas to prevent the packaging from collapsing. In meat packaging a gas mixture consisting of 60% CO_2 and 40% O_2 is common. This gives the meat an indelicately brownish color and the packaging gas is dependent on that the residual O_2 is reduced to a low level, to less than 0.2 % in order to avoid the color of meat turning grey. High oxygen gives the meat a nice red color. Disadvantages of high oxygen packaging is a shorter shelf life, more irregular color, easily exposed rancidity, and " premature browning " which means that the meat will look properly cooked by an microbiological uncertain core temperature. (Eie, 2007)

3.5.2 Vacuum packaging

In *vacuum packaging* the air is drawn out of the package with the result that in a negative pressure in the packages occurs. This method seems conservative, because the oxygen around the product is removed when the air is drawn out of the packets and then sealed. There will be a vacuum in the package, and the flexible packaging material is formed tightly around the product. Assuming a sealed container, the higher vacuum, the less oxygen is left in the package, and an O_2 sensitive product will get a longer durability. Mold, bacterial growth and most oxidizing processes will be reduced sufficiently when the oxygen concentrations is below 1-2 %. (Eie, 2007)

Vacuum packaging is used primarily for fresh and processed meat, fish products and cheese. The atmosphere in vacuum-packed fresh meat changes during the storage period. The meat produces CO_2 by breathing, which can also be produced by the micro flora, while the residual oxygen is consumed. Vacuum-packed meat, has a shorter shelf life than meat stored in pure CO_2, and products are slightly deformed by the vacuum process. (Eie, 2007)

3.6 Antimicrobials and antimicrobial packaging

Antimicrobials are usually low molecular weight molecules, natural or synthetic, that inhibits the growth of microorganisms (Zweifel & Amos 2001). Antimicrobial packaging systems were not meant to replace the need for a feasible food processing and handling, or improve poor food quality. Antimicrobial packaging can, however, protect its contents so the food retains its high quality. The slow release of antimicrobial agent can prolong shelf life and improve the food quality (Risch 2000), (Cooksey 2001).

Antimicrobial concepts have an impact on the foods surface and this can again reduce the addition of preservatives to the food. The concept consists of two main principles, *migration* – into food or foods surroundings, and *non migratory* – acts anti-microbial when adverse microorganisms touches the antimicrobial surface. For most applications direct contact between the active material and foodstuff are necessary (Eie 2007).

Antimicrobial packaging is a sort of active packaging. Antimicrobial food packaging has the ability to reduce, inhibit or retard the growth of microorganisms. It is an innovative way to inhibit microbial growth in the food while maintaining quality, freshness, and safety. Just a few food related application have been commercialized so far. Silver substituted zeolites are most widely used as polymer additives for food applications. Sodium ions are present in zeolites that are substituted by silver ions, which is antimicrobial against a wide range of bacteria and molds. (Appendini & Hotchkiss 2002)

Parameters that determine the antimicrobial activity is the concentration of the antimicrobial component, pH, temperature, type of polymer, the sensitivity of the target microorganism and the length of time the antimicrobial are in contact with the microorganisms. The use of antimicrobials as active ingredients in polymer materials have the intention to increase shelf life and give improved quality of food (Zweifel & Amos 2001).

When designing an antimicrobial packaging system, several conditions should be considered. First, the regulatory status of the antimicrobial agent is important. Second, it is important to look at the cost-to-benefit ratio; some antimicrobial agents can be effective if they are added in large quantities, but may require expenditure beyond the benefits achieved. There are also technical challenges to be considered; related to coating methods, the effects on physical and

mechanical properties of the film/laminate, the effects on color, the texture or flavor of the food, and the ability of the antimicrobial agent to provide effectiveness throughout the package/product life circle (Cooksey 2001).

Antimicrobials used in packaging material in direct contact with food, needs to fulfill special requirements to food safety. Antimicrobial packaging material cannot leach or transfer any antimicrobial additives into the food above acceptable levels or have any preservative effect on the food. Antimicrobial substances in packaging are regulated as an indirect food additive in many countries.

3.6.1 Silver ions as antimicrobial component in packaging

Silver, has non-toxicity of the active Ag+ to human cells. Silver does not release ions easily in its metallic state, and the antimicrobial activity is therefore not completely strong in this state. Among metallic ions, the silver ion has the strongest activity. Silver ions are significant antimicrobial with its antiseptic properties, and there are only a few bacteria resistant to this metal. The silver cation Ag+ is essential for the antimicrobial activity of silver ions. This cation binds strongly to electron donor groups in biological molecules containing sulphur, oxygen and nitrogen (Kumar & Münstedt 2005), (Brody et al. 2001).

Silver ions are used to prevent surface growth in food. Surface growth in food leads to a large number of spoilage and contamination. Silver ions are taken up by microbial cells and disrupting the cells' enzymatic activity. Silver ion antimicrobials have a broad range of activity against most bacteria, gram negative and likewise the gram positive. Silver ions get released through an ion exchange reaction; ions present in water are "traded" to silver ions between water medium and silver ion carriers. Silver ions are highly active when they are not bonded into carrier molecules, and they will have a fast reaction with bacteria or other substances on the surface. (WIPAK)

3.7 Meat quality and shelf life

Meat quality is ultimately defined by the consumer's acceptability. Visual characteristics, as color, textural appearance, amount of fat and visible water, meat tenderness and flavor, have a significant impact on the consumer's expectations and satisfaction of the product. Meat

quality can be measured instrumentally and/or by sensory evaluation. Microorganisms are responsible for the major part of quality loss during prolonged storage. (Nollet et al. 2012)

There are several factors that have an impact on the shelf life of meat. Storage temperature, gas composition, light intensity, packaging characteristics, which are the greatest contributors to carcass and meat contaminations. Acidity, water activity, presence of antimicrobials and identity of the natural microflora are some other factors that affects the shelf life of food (Nollet et al. 2012).

Shelf life is frequently defined as the time from production or slaughtering to unacceptability or spoilage. And hygiene at each level from raw material to finish produt. Factors affecting the shelf life on fresh meat are determined by two main factors, its color and the microbial status. A third factor is lipid oxidation, which can be important under certain conditions. The consumers expect a bright red color (oxymyoglobin) of fresh meat, brown (metmyoglobin) appears less desirable. The shelf life depends on the number and types of microorganism present and their growth, mainly bacteria. The shelf life of food depends on the environment and the atmosphere surrounding them (Warriss 2010) (Borch et al. 1996)

3.7.1 Meat color

Fresh meat and meat products are perishable and are easily exposed to color changes and microbial degradation. The protein myoglobin is color carrier in the meat. The heme-group can only bind small molecules, such as O_2, NO_2 and CO, which all provide different reds. Myoglobin has a purple color and can bind oxygen and form bright red oxymyoglobin. It may also oxidase to brown met-myoglobin, if there is plenty of oxygen present. Discoloration is affected by temperature and will occur faster at room temperature than at refrigerated temperature. In modified atmospheres of CO_2 or CO_2/N_2 can low concentrations of residual O_2 form metmyoglobin in short time, which is brown and discolored (Eie 2007).

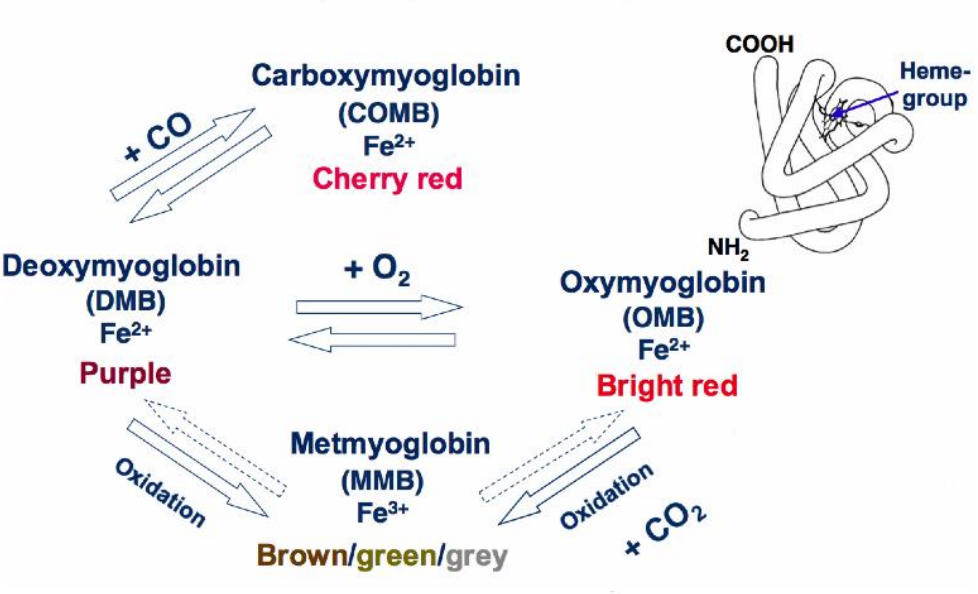


Figure 3.2 Myoglobin forms and color of meat

3.7.2 Spoilage and natural bacterial flora of fresh chilled meat

Surface growth of microorganisms is one of the leading causes of food spoilage. Natural micro-flora can eventually spoil the food or surface can be contaminated by handling during processing and packaging (Risch 2000), (Cooksey 2001).

The main groups of microorganisms are yeast, molds, gram-positive and gram-negative bacteria. The main difference between gram-positive and gram-negative bacteria is that gram-negative bacteria's has a second, outer membrane, while gram-positive bacteria's only have one membrane. Gram-negative bacteria's are therefore often less sensitive to antimicrobial than the gram-positives (Zweifel & Amos 2001).

A certain maximum acceptable bacterial level can define the point of spoilage, or an unacceptable off-odour/flavor or appearance (Borch et al. 1996). Only a few types of organisms presented in the microbial flora of meat are able to grow and will appear in the spoilage flora of meat at chill temperatures. Therefore spoilage flora of chilled meat usually contains a limited number of bacteria types. *Brochothrix thermosphacta, lactic acid bacteria,*

a few species of *Pseudomonas*, some member of the family *Enterobacteriaceae*, *Shewanella (Alteromonas) putefaciens*, *Moraxella*, and *Acinetobacter* are represented in most spoilage flora of chilled meat, depending on the initial flora and the growth environment. These different groups of spoilage bacteria can be distinguish from each other by the following properties; gram-reaction, morphology, motility, presence of catalase, oxidase, arginine dehydrolase/decarboxylase and how they utilize glucose. The growth of different spoilage bacteria can be detected by using a selective media. The bacterial population is usually underestimated, but it can be used to identify changes in the number of specific groups of spoilage bacteria (Gill & Greer 1993).

If fresh meat is exposed to microorganisms that thrive and grow in such an environment, the meat will eventually be unsuitable as food for humans. The meat will be altered in taste, smell and appearance. If the meat is kept refrigerated at a temperature between -1.5 and + 5 degrees Celsius, the microbial growth will be inhibited. And by changing the atmosphere the microbial growth are susceptible to be further reduced. Increasing the content of carbon dioxide and decreasing oxygen available can reduce the growth of most spoilage bacteria. Cold storage can prevent the growth of mesophilic (cold intolerant) species, and ensure that only the few psychrotrophic (cold tolerant) organisms present in meat are able to grow (Gill & Greer 1993).

Physical requirements for bacterial growth are temperature and pH. Most bacteria grow only within a limited range of temperature. Microorganisms are classified into three primary groups on the basis of their preferred range of temperature. Psychrophiles (cold-loving), Mesophiles (moderate temperature-loving) and Thermophiles (heat-loving). pH, the acidity or alkalinity of the food, as growth material. Most bacteria grow best between pH 6.5-7.5 and very few grow at a pH below about 4 (Tortora et al. 2007).

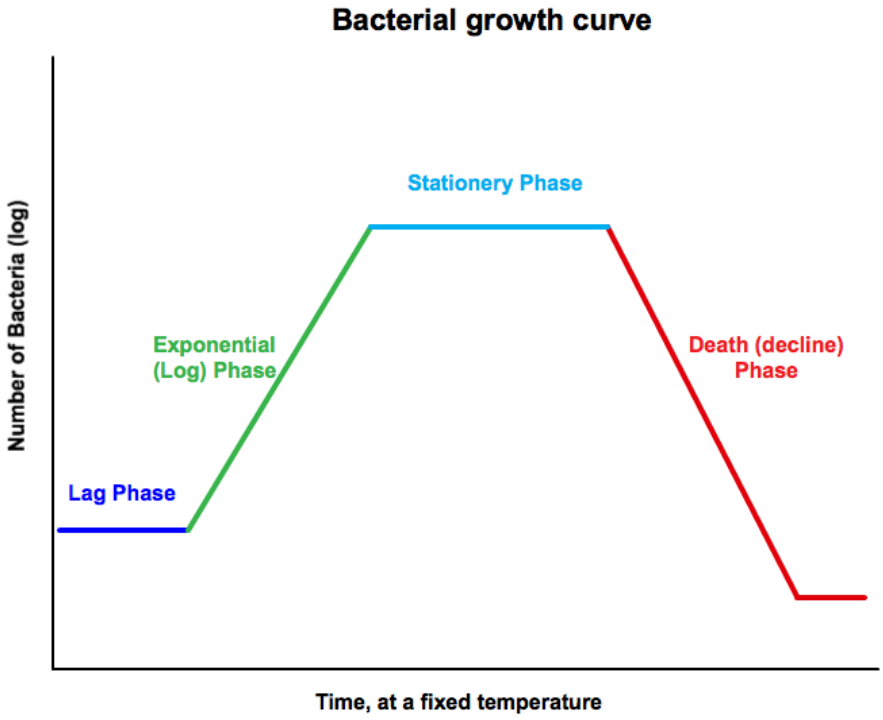


Figure 3.3 Bacterial growth curve, showing the four typical phases of growth

The growth of bacteria is a logarithmic scale with four phases: lag, log, stationary and death phase. Bacteria grow by each cell dividing into two daughter cells.

1. The lag phase; little or no cell division. The number of cells changes very little – because the cells do not immediately reproduce in a new medium.
2. The log phase; cells begin to divide, the cellular reproduction is most active in this period.
3. The stationary phase, a period of equilibrium. The growth rate slows down; the number of microbial deaths balances the number of new cells.
4. The death phase; the number of death exceeds the number of new cells formed.

Different microorganisms have different requirements for the amount of O_2. And can be divided into three groups depending on the O_2 requirements. A complete aerobic bacterium requires O_2. Facultative anaerobic bacteria can grow with or without O_2 (*lactic acid bacteria*). And complete anaerobic bacteria require an oxygen-free environment (Tortora et al. 2007).

Meat exposed to air, stored at chill temperatures are dominated by species of *Pseudomonas*, and other strictly aerobic genera like *Acinetobacter* and *Moraxella*. *Enterobacteriaceae* and *Brochothrix thermosphacta* may also be present (Gill & Greer 1993).
Pseudomonas, *Enterobacteriaceae*, *Lactic acid bacteria* and *Brochothrix thermosphacta* which are the different groups of spoilage bacteria that have been detected in this research, will be further mentioned.

Table 3.2 Differentiation of the principle bacteria found on chilled meat. (*Pseudomonas*, *Enterobacteriaceae*, *Lactic acid bacteria* and *Brochothrix thermosphacta*).

	Gram reaction	Morphology	Catalase	Oxidase	Arginine metabolism	Glucose metabolism	Motility
Pseudomonas	-	Bacilli	+	+	+	0/-	+
Enterobacteriaceae	-	Short bacilli	+	-	-/+	F	+/-
Lactic acid bacteria	+	Bacilli/cocci	-	-	+/-	F	-
Brochothrix thermosphacta	+	Bacilli	+	-	-	F	-

- Negative reaction, + positive reaction, 0 oxidative, F fermentative

Pseudomonas is gram-negative motile rods. They are strictly aerobe, which means that they only will growth with O_2 present. *P. fragi* and *P. flourescens* are the main species of *Pseudomonas* which will dominate the aerobic spoilage flora of chilled meat. Growth of *pseudomonas* can be inhibited by removing O_2 and/or using high CO_2 concentrations. Spoilage occurs when bacterial numbers exceed $10^8/cm^2$, and when bacterial number approach $10^9/cm^2$ slime becomes visible on the meat surface (Gill & Greer 1993).

Enterobacteriaceae are gram-negative rods, that ferment sugars which usually leads to gas production. The *Enterobacteriaceae* family consists of a variety of facultative anaerobic organisms, which include some pathogenic species. This group of bacteria contributes rarely in the aerobic spoilage flora, because their aerobic growth are slow compared to the growth of *pseudomonas*. *Enterobacteriaceae* are therefore more important in anaerobic conditions like vacuum-packed chilled meat for instance (Gill & Greer 1993).

Lactic acid bacteria consist of gram-positive rods and cocci, which is typically non-motile. They are facultatively anaerobic, which usually dominate in the flora of meat stored anaerobically. *Lactic acid bacteria* can be homo- or heterofermentative. Homofermentative

bacteria are producing lactic acid as the main product of glucose fermentation, while heterofermentative bacteria produce a mixture of lactate, carbon dioxide and ethanol from glucose (Gill & Greer 1993).

Brochothrix thermosphacta is gram-positve bacteria that are non-motile. This bacteria group are facultative anaerobic that occur in the flora of meat stored in air and in vacuum packages. If the pH is above 5,8 it is of greater importance in anaerobic than in aerobic spoilage flora. Will not grow anaerobically at pH values below 5,8 (Gill & Greer 1993).

4. Materials and Methods

4.1 The meat samples

The meat samples in this research were all taken from beef loin steaks/ sirloin from cattle, distributed by Nortura, Rudshøgda and pre-tenderized to the 4th, 5th and 6th of February 2014. The cattle was most likely slaughtered between the 21th and 24th of January 2014, because carcass normally hang 48 hours after slaughtering before cutting and packing, and the tenderization of meat takes approximately 10 days.

4.2 Packaging

250 grams pieces of meat was weighted out for the sample packages on a digital balance (PC16, Mettler, Switzerland), more than 30 kg of beef was needed for this research. The meat samples were packed according to three different packaging methods, and in two different types of packaging material – combinations of polymers/laminates, which gave 6 varieties of packaging. At each sample point 4 parallels of each varieties was analyzed, and in total there were five sample points which gives the following equation: 6 varieties multiplied with 5 sample points, multiplied with 4 samples taken out from each varieties which is equal to 120 meat sample packages (6 x 5 x 4 = 120). It was necessary to have more than 120 meat packages – at least 5 extra of each variety, in case of damage or leakage.

After cutting, finding the correct weight an packing method, the individually samples were stored in a dark chilling room at 4 °C up to 30 days. Approximately once a week during the period, 4 meat samples of each variety of packaging were taken out from the chilled storage to be analyzed. The sampling points were after 9, 16, 22, 27 and 30 days of storage.

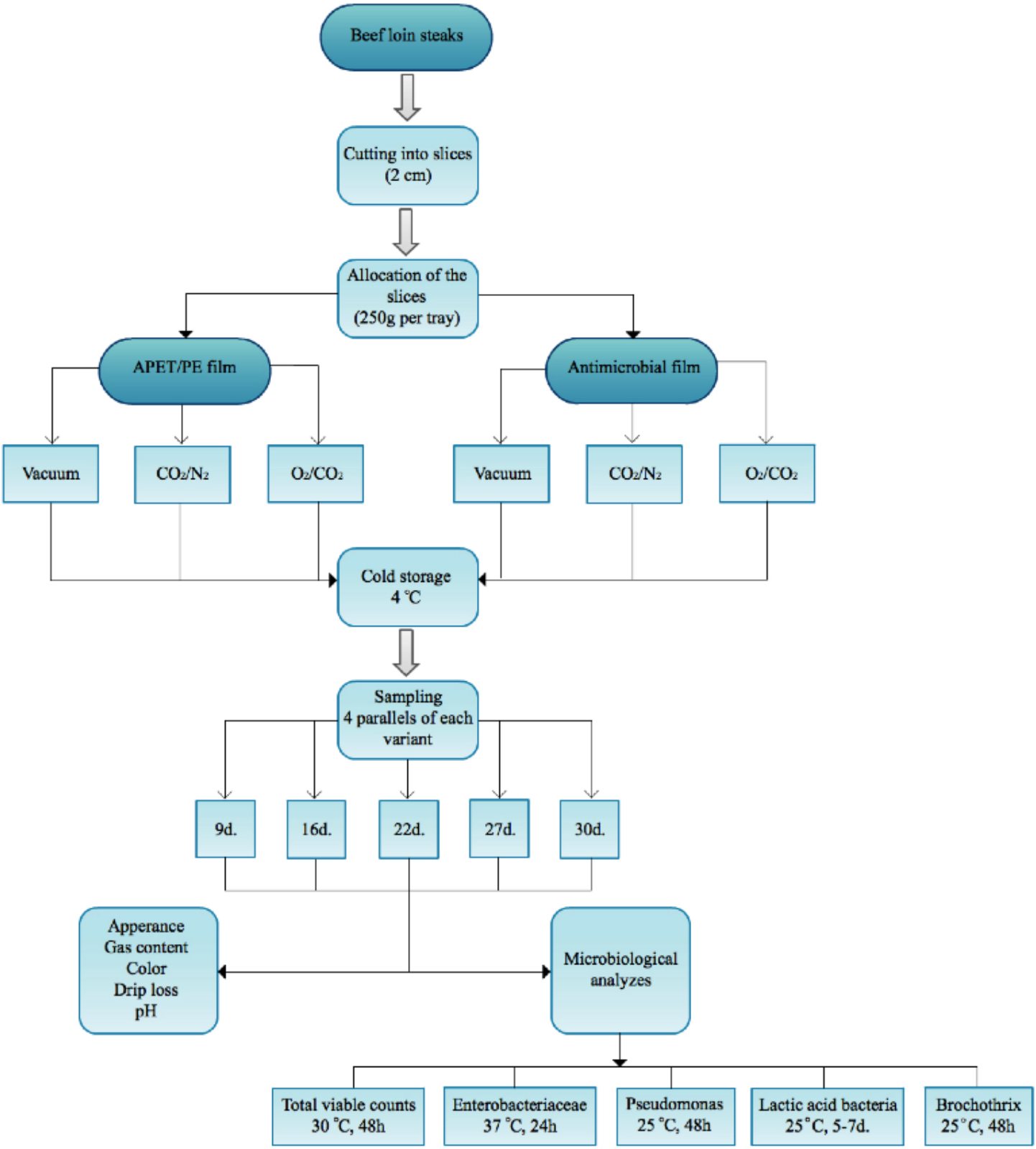


Figure 4.1 Flow chart of the storage experiment

4.1 Packaging material and method

4.1.1 Packaging material

Half of the samples were packed with an antimicrobial film consisting polyamide, ethylene vinyl alcohol and polyethylene. The other half of the samples were packed with amorphous polyethylene terephthalate and polyethylene laminate (APET/PE). The antimicrobial film was incorporated/integrated with silver ions as the antimicrobial component, and was made with the following layers; polyamide layer on both sides of the ethylene vinyl, an alcohol layer and a polyethylene layer on top of one of the polyamide layers (PA/EVOH/PA/PE). The silver ions in this polymer are bounded into larger carrier molecules that keep them stable until time of use. The active antimicrobial silver particles in this solution are not nanoparticles. The carrier particles of silver ions have an average particle size of 2µm. Nanoparticles are particles that range from 1 to 100 nanometers in diameter. Liquid absorber pads was used in all samples.

Table 4.1 Thickness and oxygen transmission-properties for each material used in this research.

Material	Structure	Thickness	Oxygen transmission	Producer
"Regular" Top film	**BOPET/PE** Amorphous polyethylene terephthalate/ Low density polyethylene	65µm	5	WIPAK OY
"Regular" Bottom film	**APET/PE** Amorphous polyethylene terephthalate/ Low density polyethylene	540µm	7	WIPAK OY
Antimicrobial Top film	**PA/EVOH/PA/PE** Polyamide/ Ethylene vinyl alcohol/ Polyamide/ Low density polyethylene	90µm	<4	WIPAK OY
Antimicrobial Bottom film	**PA/EVOH/PA/PE** Polyamide/ Ethylene vinyl alcohol/ Polyamide/ Low density polyethylene	140µm	<2	WIPAK OY

4.1.2. Packaging machine and method

A thermoforming machine (R145, Multivac, Germany) was used in this project. As mentioned in the theory, a thermoforming machine produces packaging by a bottom film and a top film where the bottom film is thermoformed. The Functional principles; mold tool, prior forming, load zone, welding tool. In the welding tool the air is removed from packaging, or supplied a gas mixture - in our case 70% O_2 and 30% CO_2 or 60% CO_2 and 40% N_2

4.2 Measurements and analysis

At all sample points, 4 parallels of each variant was measured

Analysis used to evaluate meat quality and durability of the different varieties:

- Gas composition
- Measuring color of the product with MINOLTA – color meter
- Drip loss
- Microbiological analysis – detect bacterial growth on petri dishes
- pH – to measure the acidity and look at differences between varieties and changes over time. (Indicator of eating quality)

4.2.1 Gas analysis

Gas composition/content was measured by using a O_2, O_2/CO_2 headspace analyzer (CheckMate 9900, PBI-Dansensor A/S, Denmark 2004). Oxygen (O_2) and carbon dioxide (CO_2) concentration in the package headspace were monitored in percent by sampling 3 ml of gas from the package headspace with a syringe needle. Rubber/septa sheets, 20 x 200 mm, cut into smaller pieces were attached to the packaging samples, to prevent any leakage of gas.

4.2.2 Colorimetric measurement

Surface color was measured by a chroma meter with a circular measurement area (CR-400, Konica Minolta, Japan). It was used to analyze the difference in color between the different varieties and color changes over time. It gives us an L*-, a*- and b*-value, for each samples. L* shows lightness, a* redness and b* yellowness. A low a*-value tell us that the sample is

more green and a low b*-value that is more blue. The colorimeter was connected to a computer-with a program where it easily can be controlled and name the measurements. All the measured values appeared in a table, which was transferred to an excel sheet after measuring all the samples. Since the colorimeter was not used direct on the meat, but trough the top film material. The colorimeter was calibrated using a white standard plate with a piece of the specific film on top of it.

4.2.3 Drip loss

Drip losses were measured by weighing each packaging before and after sample points using a balance (MS3002S, Mettler Toledo, Switzerland). The meat packaged was weighted right after packaging (start point) and empty packaging were weighted after each storage time (sample point after removal of all the meat); were only the pad and liquid are left in the packaging – in order to find drip loss.

Equation for the drip loss of each sample is as following:
Drip loss % = (Weight gain of liquid absorber pad + any visible liquid / Starting weight, after packaging) x 100

4.2.4 Microbial analysis

Microbiological analysis was used to check the bacterial growth on the surface of the different meat samples. This research project looked for the growth of *Enterobacteriaceae*, *Lactic acid bacteria* (LAB), *Brochothrix*, *Pseudomonas* spp., which is a typical spoilage bacterium on meat. The total number of bacteria was also detected with plate count agar.

4.2.4.1 Making Agar

Different types of agar need to be made in advance to detect the growth of different type of bacteria. Plate count agar, PCA (CM0463, OXOID, UK), Violet Red Bile Glucose agar, VRBGA (CM0485, OXOID, UK), The man, Rogosa and Sharpes agar, MRS (CM0361, OXOID, UK), Streptomycin Thallus Acetate Acidione agar, STAA (CM0881, OXOID, UK) and *Pseudomonas* agar base, CFC (CM0559, OXOID, UK).

The recipe on the container was used to make the different types of agars. It says how much agar powder that needs to be added to 1L or 0.5L. Bottles of 1- and 0.5 liters, and precision balance (XS6001S, Mettler Toledo, Switzerland) was used. The bottles were never filled straight up, to prevent the agar to boil over. A calculation was needed to get the right amount. The weighted agar powder was put in to the bottle and then ion exchange water was added, and put in a certoclav (EL sterilizer, CertoClav, Austria 2006). A certoclav is a compact and fast autoclave for laboratory use. The surroundings were sterile when the agar was poured into petri dishes. CFC and STAA, glycerol and a specific supplement were also added, as initiated on the container. CFC supplement (SR0103E, OXOID, UK) and STAA supplement (SR0151E, OXOID, UK)

Glycerol was added before autoclaving, while the specific supplement was added after. These agar bottles had to cool down to a temperature of (°C) degrees before adding the supplement. They were put into a water bath, to achieve a gradual reduction in temperature and to prevent coagulating. After more than an hour, the agar supplement was added. But before adding it to the bottle of agar the supplement had to be added sterilized water and/or rectified spirits (rectified spirits, Arcus kjemi AS, Norway) depending on if it is the CFC supplement or the STAA supplement, and for this a pipette controller (Accu-jet pro, Brand GMBH + CO KG, Germany) and 5 ml serological pipet, standard tips was used.

On the MRS-container it says *boiling for 15 min at 121 ºC*, but MRS contains a lot of sugar and therefor more sensitive to heat. Because it rapidly would turn brown, a boiling temperature of 115 ºC was used instead. VRBGA was made shortly before use, at sample point, because it has to be used within a short amount of time. VRBGA was used for embedding, which means that it was poured into petri dishes after adding the samples.

4.2.4.2 Preparation of the sample for microbiological analysis on medium

Meat pieces of 10 gram+ or of approximately 3 x 3 x 1cm, was cut out with a scalpel from each sample-slice and put in a blender bag with side filter (BBAG-04, VWR, USA).
The scalpel blade was replaced regularly, burned off between every sample by a bunsen burner (Fireboy plus, Integra bioscience, Switzerland). The samples in blender-bags were diluted 1:10 with peptone-water, with a dilution automate (Dilumat 3 MK2 – AESAP 1055, AES Laboratorie, France 2008) that dilutes based on the weight. The bags are then placed in a

sample homogenizer (smasher lab blender – AESAP 1064, AES Laboratorie, France 2008) were they are being smashed/homogenized for 60 seconds at room temperature. After that approximately 10 ml of the solution was transferred to a 14 ml falcon round-bottom tube (PEF 352059, Becton, Dickinson and Company, USA) with a 10 ml serological pipet, standard tips. A marker was used to mark blender bags, test tubes with sample number. And the petri dishes with sample number, agar type, dilution and date.

Equipment such as gloves, pasteur pipettes, spore spreader, sterile stick, serological pipet – standard tips, tissues, pipette tips was produces by VWR international, USA.

4.2.4.3 Dilution of samples

Dilution series were also made, depending how high dilution was needed. An increased growth of bacteria over time was expected, and higher dilutions was needed to get secure and countable values. The dilutions where made by adding 500µl of the sample with the use of a finnpipette (4500 200-1000 µl, Thermo fisher scientific, USA) in to a sterilized test tube, with 4,5ml peptone water. The dilutions where mixed with a
digital shaker (MS 3 – with standard attachment (MS 3.1), IKA, Germany) between each transfer of sample.

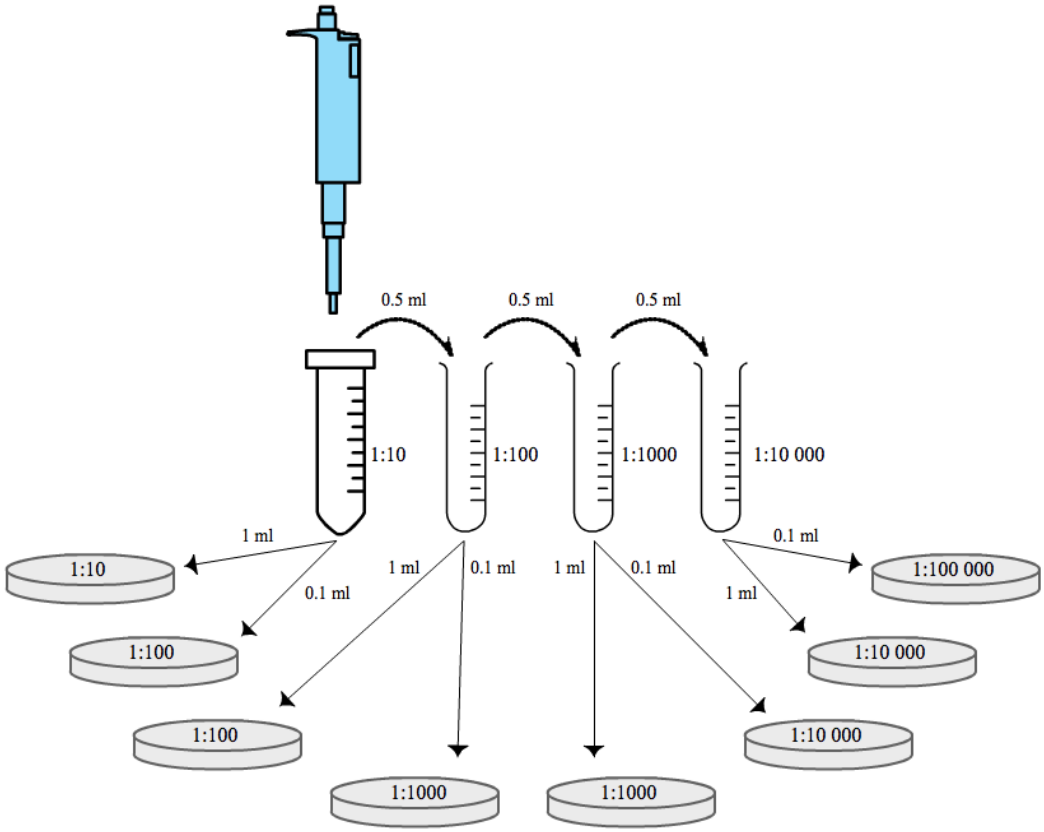


Figure 4.2 Dilution of samples

4.2.4.4 Pour- and spread plating

Different aliquots of the appropriate dilution were spread onto the following media; PCA, VRBGA, MRS, STAA and CFC, with the use of a finnpipettes for 1000/500µl (4500 200-1000 µl, Thermo fisher scientific, USA) or 100µl (4500 20-200 µl, Thermo fisher scientific, USA). A spore spreader was used to spread spores manually, and a Whitley automatic spiral plater – WASP (WB03TJ, Don Whitley scientific, UK) was used to spreads spores automatic after transferring the sample. Each sample was transferred to a small cup that was placed where the instrument would souk up the sample. An electronic laboratory vacuum pump (Whitley vacuum source 602, Don Whitley scientific, UK) was connected to the WASP. A sterile bench with a fan was used for drying the sample material on the different agars and pouring VRBGA into the petri dished after adding the samples. After drying they were stored aerobic in laboratory incubators (B8000, Termaks, Norway) with different temperatures, depending on the type of agar/detection. Further information about incubations temperature and storage time for each medium are given in the table below.

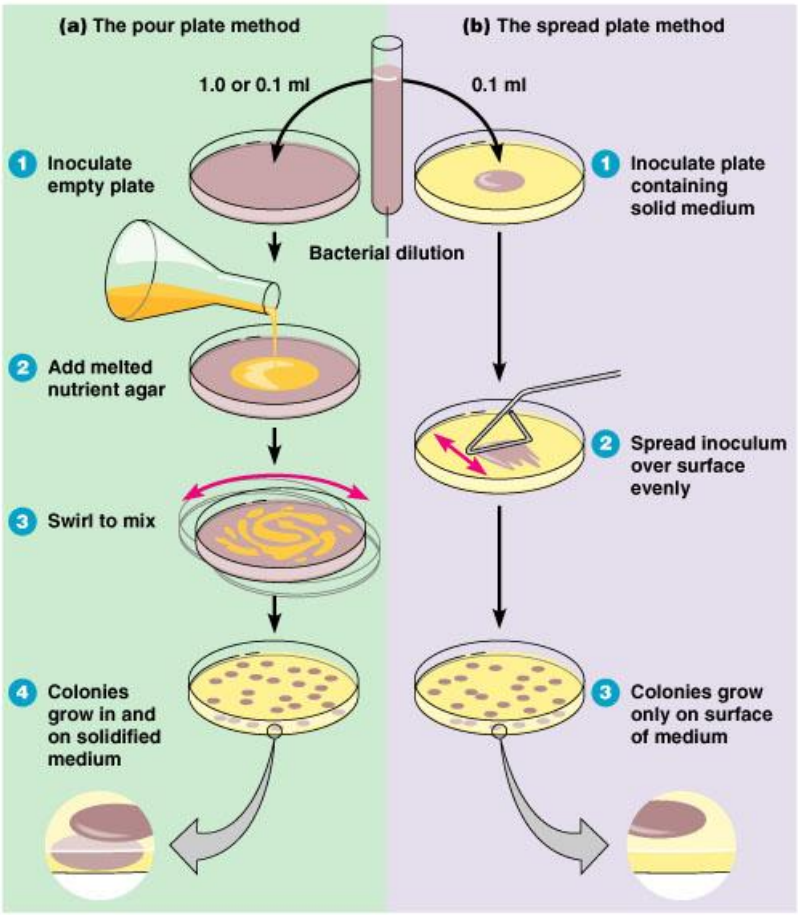


Table 4.2 Basic information of different medium which have been used for detection of total number, *Enterobacteriaceae*, *lactic acid bacteria*, *Brochothrix* and *Pseudomonas ssp*. Information as name, abbreviation, what it detects, storage temperature and storage time.

Medium	Abbreviation	Detect	Storage temp.	Storage time
Plate count agar	PCA	Total number, (Mesophilic aerobic bacteria)	30°C	3 days
Violet Red Bile Glucose agar	VRBGA	*Enterobacteriaceae*	37°C	24h
The man, Rogosa and Sharpes agar	MRS	*Lactic acid bacteria (LAB)*	25°C	5-7 days
Streptomycin Thallus Acetate Acidione agar	STAA	*Brochothrix*	25°C	48t
Pseudomonas agar base	CFC	*Pseudomonas ssp.*	25°C	48t

4.2.4.5 Counting of colonies and Oxidase test

After the specific incubation time for each "medium", the plates that was spread manually were also counted manually with or without the use of a marker. The spiral plates which was spread with WASP, high-resolution automated colony counter (Protocol 2, Synbiosis, UK) was used. To determine whether there is a growth of
monas on the CFC-plates, an oxidase test have to be performed. This is done by the use of sterile stick for picking colonies and transferring it to oxidase paper. If the paper turns blue, the oxidase test has been positive, which means that this colony is *pseudomonas*.

4.2.5 pH

pH were measured with a pH-meter (PHI31, Beckman, USA). When cutting out the samples from meat slices for microbiological analysis, the rest of the slice were put in plastic bags marked with their sample number. The samples were kept in the refrigerator and measured in the end of the day. Before measuring the sample, the pH-meter was calibrated with a 7-pH buffer solution (TEP7, WTW, Germany) and a 4-pH buffer solution (TEP4, WTW, Germany). Distillated water was used to rinse the electrode between the samples, and it was used tissues to blot dry the electrode.

4.2.6 Statistical analysis

1. General Linear Model (GLM) in Minitab

There were carried out a GLM for all the results of the samples. One with and one without parallel as a factor. Parallel was included as a factor to exclude that there are greater differences between the parallel than between the different samples. GLM was done to examine which factors that had a effect, and gave significant difference between samples.

2. One-way ANOVA in Minitab

A One-way ANOVA was done to detect significant differences of the responses between samples. The different responses after 9, 16, 22, 27 and 30 storage days, versus packaging material and gas mixture was analyzed with One-way ANOVA.

5. Results

5.1 Gas concentrations

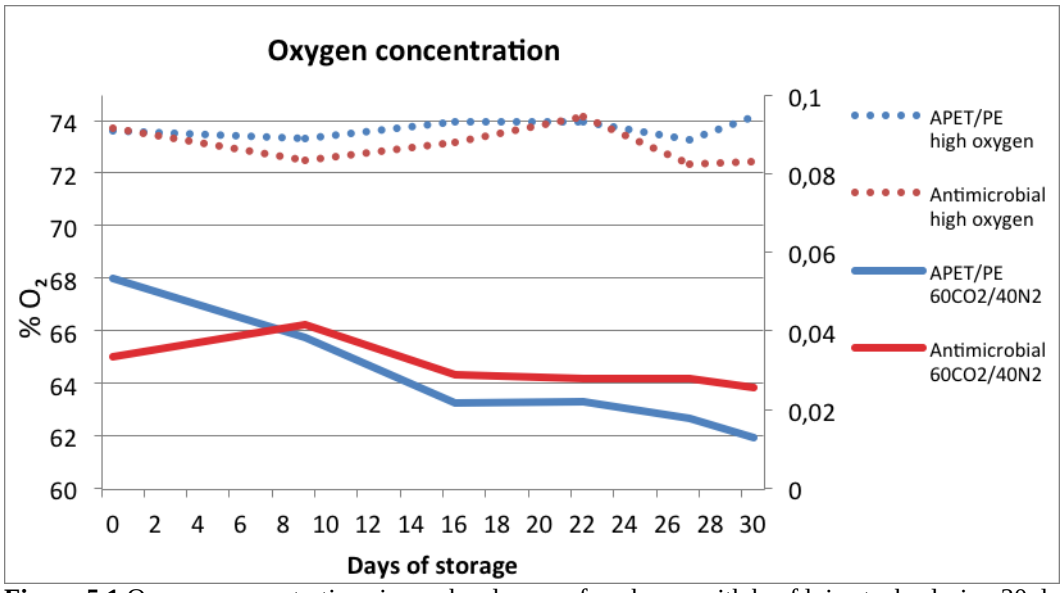


Figure 5.1 Oxygen concentrations in gas headspace of packages with beef loin steaks during 30 days of storage at 4 °C. The **red** lines show the concentration in antimicrobial packaging and the **blue** lines shows the concentrations in APET/PE packaging. Dotted lines illustrate the content in high oxygen samples. The solid lines illustrate packages packed with a gas mixture of 60% CO_2 and 40% N_2.

The graphs in figure 5.1 illustrates O_2 content in different packaging materials packed with the two different atmospheres; high oxygen and 60% CO_2 and 40% N_2, during a storage period of 30 days. There are just minimally changes in most samples. In the samples packed with high oxygen, the CO_2 content have a variation from 73,3-74,2 % for APET/PE packaging material, and 72.4 – 74,2% for the antimicrobial packaging material. To the samples packed in modified atmosphere, 60% CO_2 and 40% N_2, also indicate a small variation of O_2 content, from 0,015-0,050 % for APET/PE packaging material, and 0,025-0,042% for antimicrobial packaging material. It has been a small decrease in O_2 concentration during the storage period for the samples packed with modified atmosphere of 60% CO_2 and 40 N_2. The O_2 content in the APET/PE packaging has only had a small decreased, while the O_2 content in the antimicrobial packaging increased slightly the first 9 days, and then had a decrease as well.

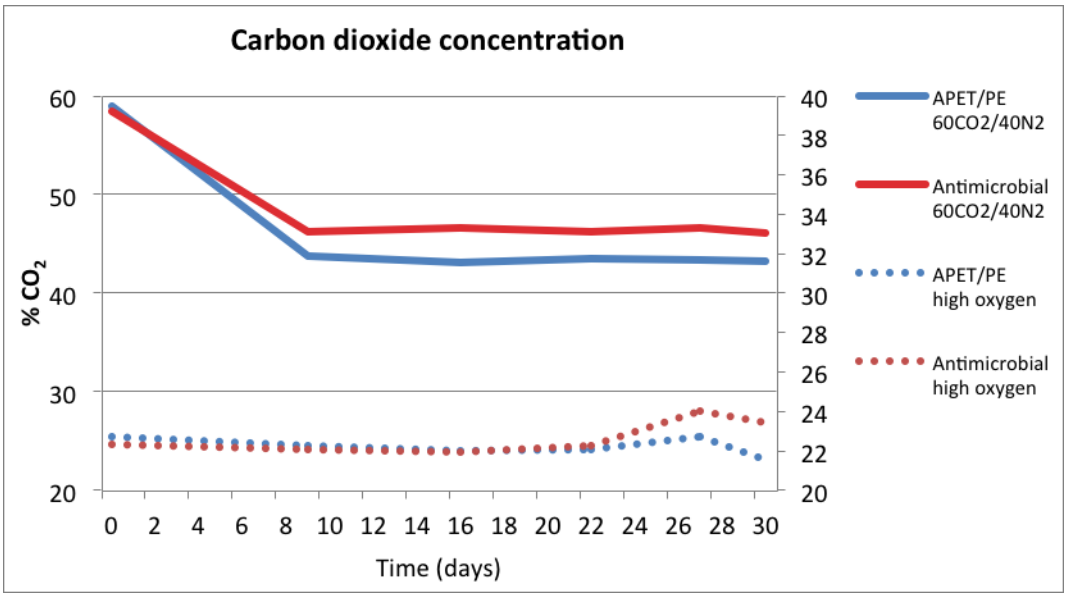


Figure 5.2 Carbon dioxide concentrations in gas headspace of packages with beef loin steaks, during 30 days of storage at 4 °C. The **red** lines show the concentration in antimicrobial packaging and the **blue** lines shows the concentrations in APET/PE packaging. Dotted lines illustrate the content in high oxygen samples. The solid lines illustrate packages packed with a gas mixture of 60% CO_2 and 40% N_2.

The graphs in Figure 5.2 shows the CO_2 content in different packaging materials packed with the two different atmospheres; high oxygen and 60% CO_2 and 40% N_2, during a storage period of 30 days. There are almost no changes in the CO_2 content for the sample packed in high oxygen, except a small increase from day 22. After day 27 the CO_2 content in these samples has decreased again to similar levels as before the increase. The content varies from 21,6-2,7 % for R, and 22-24 % for A. There has been an overall decrease of the CO_2 concentration during the storage period, of the samples packed in 60 % CO_2 and 40 % N_2. It was a sharp decline from start to day 9, with a drop from 59 % to 43,8% for the APET/PE packaging material and 58,4 % to 46,1% for the antimicrobial packaging material. After this the CO_2 content has been almost "constant" in both packaging variants.

4.2 Color changes

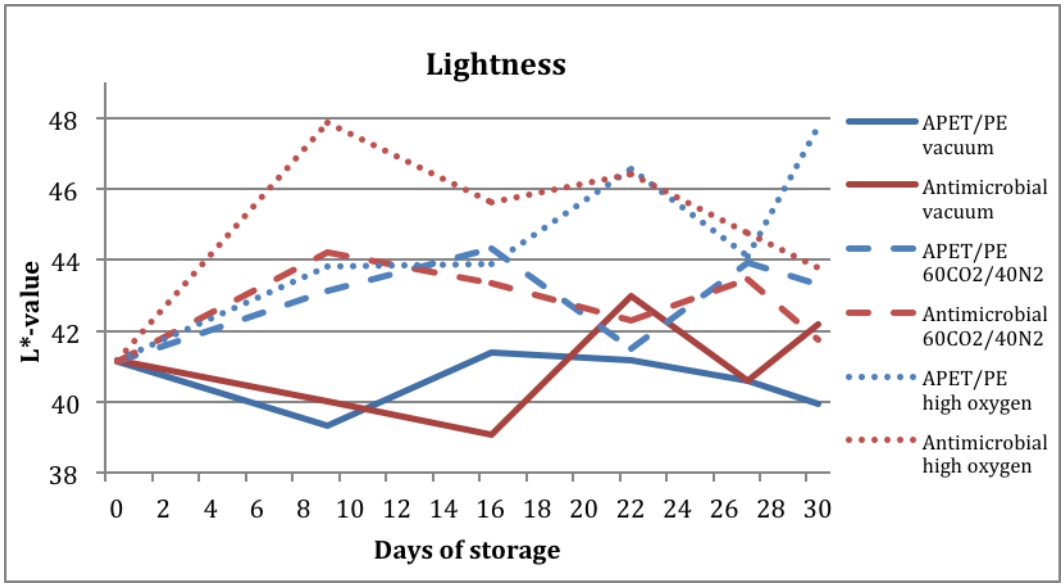


Figure 5.3 The L* value on beef loin steaks during 30 days of storage at 4 °C. The **red** lines show the concentration in antimicrobial packaging and the **blue** lines shows the concentrations in APET/PE packaging. Dotted line: vacuum, dashed line: high oxygen (75% O_2/25% CO_2), and solid line: MAP (60% CO_2/40% N_2)

As shown in Figure 5.3, the L* value was highest for meat samples packaged with high oxygen, and lowest for vacuum packaged meat. The high oxygen samples have a lighter hue, compared to packages with a gas mixture of 60% CO_2 and 40% N_2, and especially vacuum packaged meat.

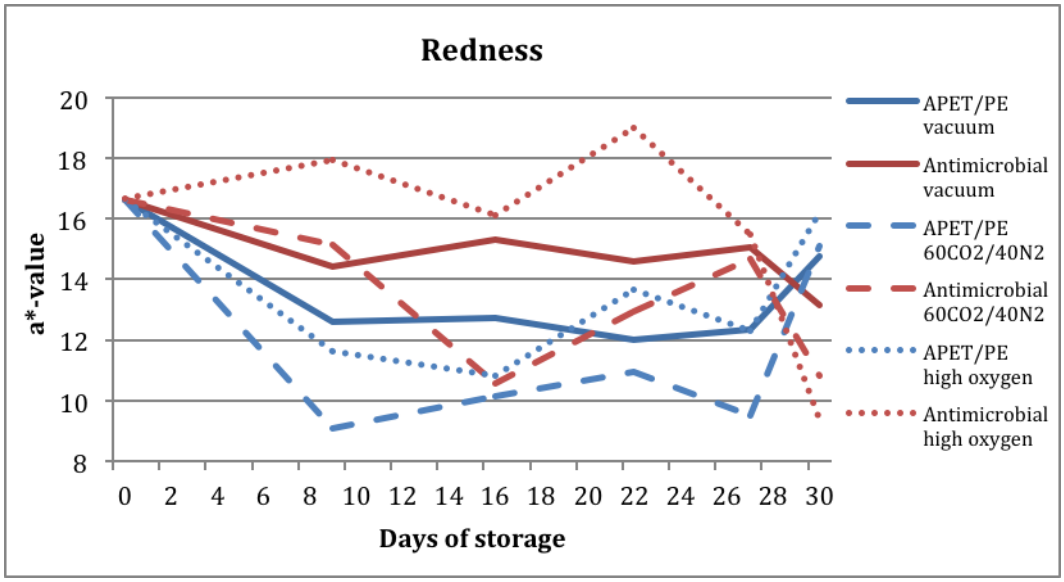


Figure 5.4 The a* value on beef loin steaks during 30 days of storage at 4 °C. The **red** lines show the concentration in antimicrobial packaging and the **blue** lines shows the concentrations in APET/PE packaging. Dotted line: vacuum, dashed line: high oxygen (75% O_2/25% CO_2), and solid line: MAP (60% CO_2/40% N_2)

The antimicrobial samples, Figure 5.4, has a higher a* value than the APET/PE samples with the same packaging method/atmosphere. This means that the antimicrobial high oxygen sample for example has redder hue, compared to the APET/PE high oxygen sample.

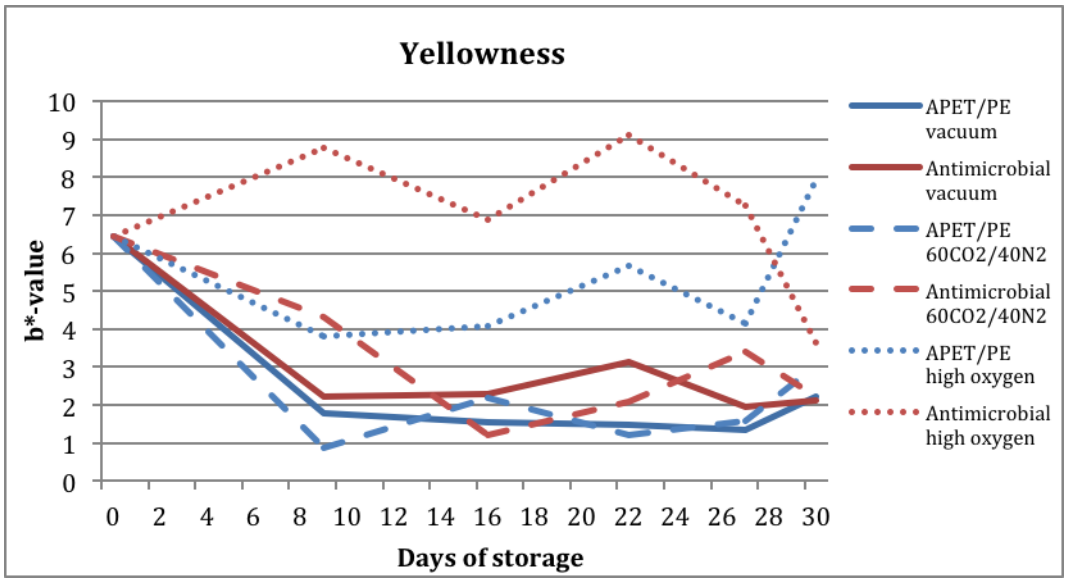


Figure 5.5 The L* value on beef loin steaks during 30 days of storage at 4 °C. The **red** lines show the concentration in antimicrobial packaging and the **blue** lines shows the concentrations in APET/PE packaging. Dotted line: vacuum, dashed line: high oxygen (75% O_2/25% CO_2), and solid line: MAP (60% CO_2/40% N_2)

The high oxygen samples have the highest b* value, and have a more yellow color tint compared to the rest of the samples, Figure 5.5. APET/PE high oxygen has also a relatively high b* value.

5.3 Drip loss

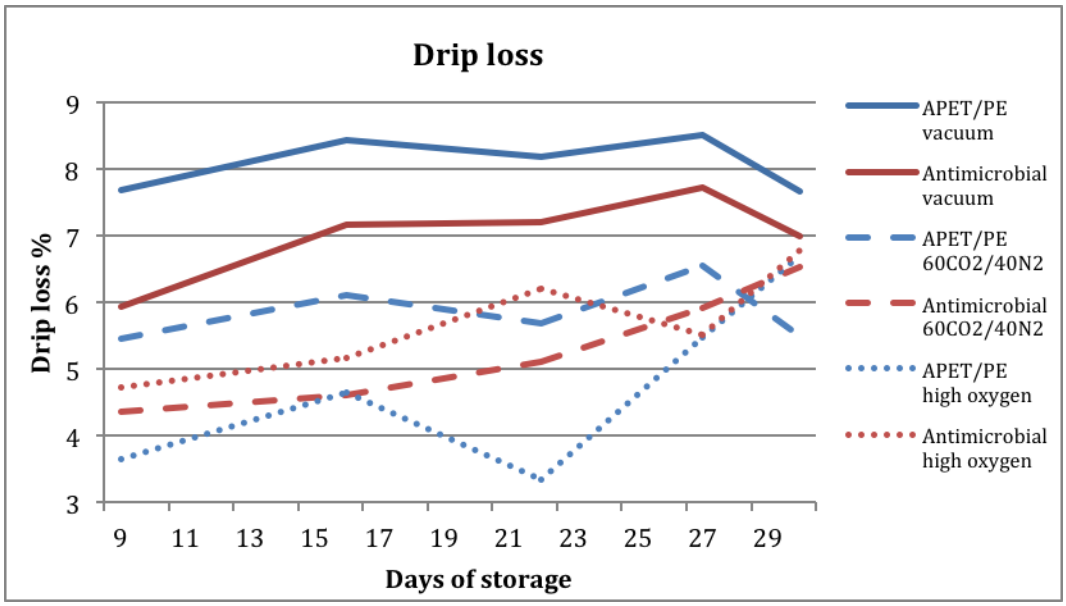


Figure 5.6 The drip loss in beef loin steaks during 30 days of storage at 4 °C. The **red** lines show the concentration in antimicrobial packaging and the **blue** lines shows the concentrations in APET/PE packaging. Dotted line: vacuum, dashed line: high oxygen (75% O_2/25% CO_2), and solid line: MAP (60% CO_2/40% N_2)

This graph, Figure 5.6, indicates that the % drip loss was higher for vacuum packaged meat compared to the other packaging methods, and highest in APET/PE vacuum packaging.

5.4 Microbial growth

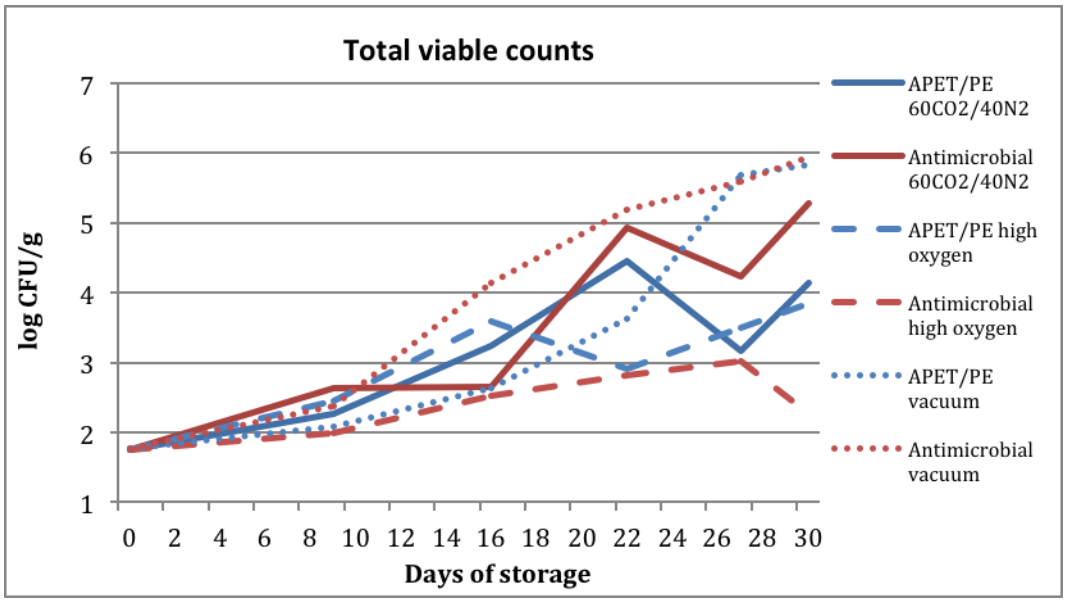


Figure 5.7 The changes in total viable counts (log CFU/g) on beef loin steaks during 30 days of storage at 4 °C. PCA-agar was to detect the total number of mesophilic aerobic bacteria, incubated at 30 °C in 3 days. The **red** lines show the growth on meat in antimicrobial packaging and the **blue** lines shows the growth on meat in APET/PE packaging. Dotted line: vacuum, dashed line: high oxygen (75% O_2/25% CO_2), and solid line: MAP (60% CO_2/40% N_2)

The graph in Figure 5.7, illustrate that the total number of mesophilic aerobic bacteria has increased during the storage period. The total number for samples packed in vacuum has increased throughout the storage period. Antimicrobial packaging with vacuum had the highest growth mesophilic aerobic bacteria early compared to the other samples. Antimicrobial packaging with high oxygen had the lowest growth of mesophilic aerobic bacteria and had also a decline in the end, from day 27 to day 30, of the storage period.

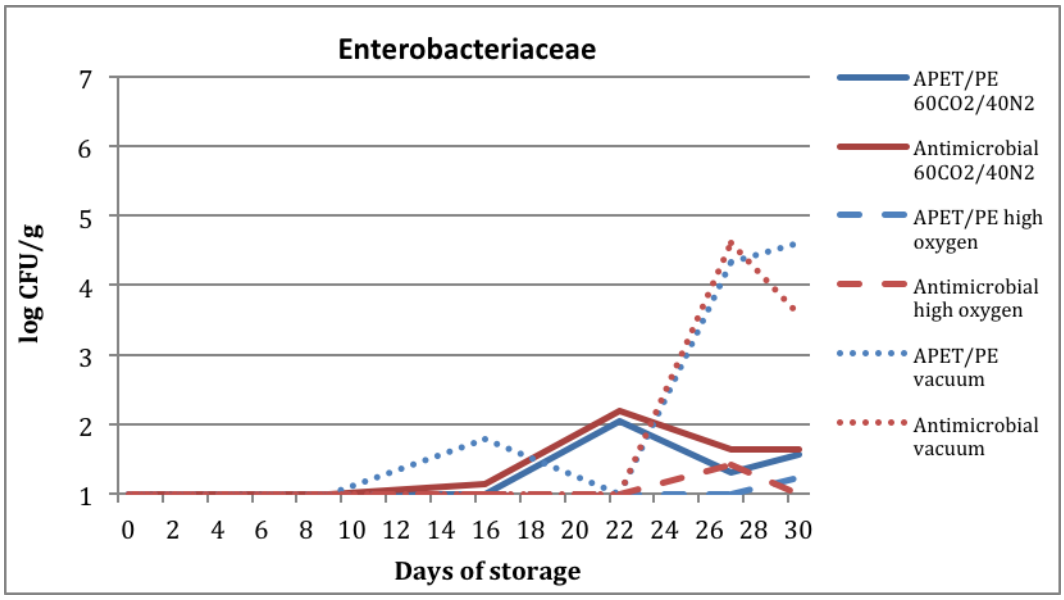


Figure 5.8 The changes in growth of *Enterobacteriacea*e (log CFU/g) on beef loin steaks during 30 days of storage at 4 °C. The growth of *Enterobacteriaceae* was detected by the use of VRBGA-agar incubated at 37 °C in 24 hours. The **red** lines show the growth on meat in antimicrobial packaging and the **blue** lines shows the growth on meat in APET/PE packaging. Dotted line: vacuum, dashed line: high oxygen (75% O_2/25% CO_2), and solid line: MAP (60% CO_2/40% N_2).

The line that shows the growth of *Enterobacteriaceae* on antimicrobial vacuum samples, are behind the line that show the growth of *Enterobacteriaceae* on antimicrobial high oxygen samples. This two samples, has had the exact same growth.

The samples packed with vacuum and with APET/PE packaging material had the highest growth of *Enterobacteriaceae*, Figure 5.8. The samples with a high oxygen atmosphere, which include both varieties of samples – the ones with APET/PE packaging material, and the ones with antimicrobial packaging material; had the lowest growth of *Enterobacteriaceae*. This also includes vacuum packaged meat with antimicrobial packaging material. The red short dotted line that shows the growth of *Enterobacteriaceae* on antimicrobial vacuum samples, are behind the red dotted line that show the growth of *Enterobacteriaceae* on antimicrobial high oxygen samples. This two samples, has had the exact same growth.

The meat packed in modified atmosphere, 60% CO_2 and 40% N_2, had similar growth of *Enterobacteriaceae* on both varieties, but the ones with antimicrobial packaging material had slightly higher growth. The meat packed in high oxygen atmosphere also had a similar growth of *Enterobacteriaceae* on both varieties. It was larger differences in growth of *Enterobacteriaceae* between the two vacuum packed varieties.

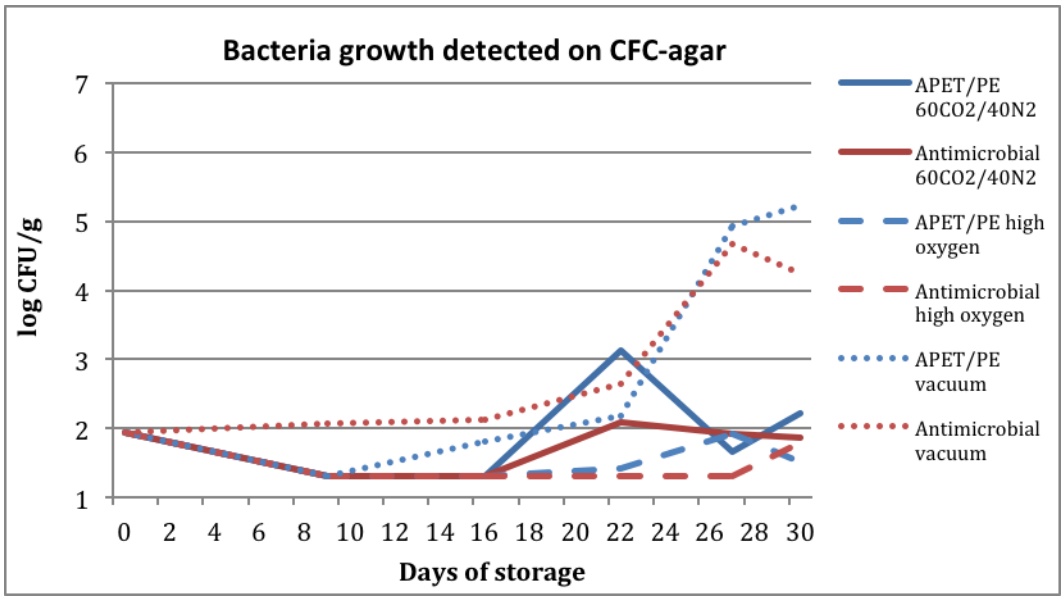


Figure 5.9 Growth of bacteria (log CFU/g) on beef loin steaks during 30 days of storage at 4 °C, which was detected on CFC-agar. Incubated at 25 °C in 48 hours. The **red** lines show the growth on meat in antimicrobial packaging and the **blue** lines shows the growth on meat in APET/PE packaging. Dotted line: vacuum, dashed line: high oxygen (75% O_2/25% CO_2), and solid line: MAP (60% CO_2/40% N_2)

Figure 5.9, shows larger differences in growth of *Pseudomonas* between the same packaging methods (but different packaging material), compared to the growth of *Enterobacteriaceae*. But there is a similar growth between the samples with the same packaging method. Vacuum packed meat samples had greatest growth of *Pseudomonas*, while high oxygen meat samples had a minimum growth of *Pseudomonas*.

In the graphs that show the growth of *Enterobacteriaceae* and *Pseudomonas* spp. similar pattern can be seen. The oxidase tests (except one/once) did not give any positive results, which may indicate that both graphs shows growth of *Enterobacteriaceae* – since it was detected growth at nearly the same samples on both agar types and with similar amounts.

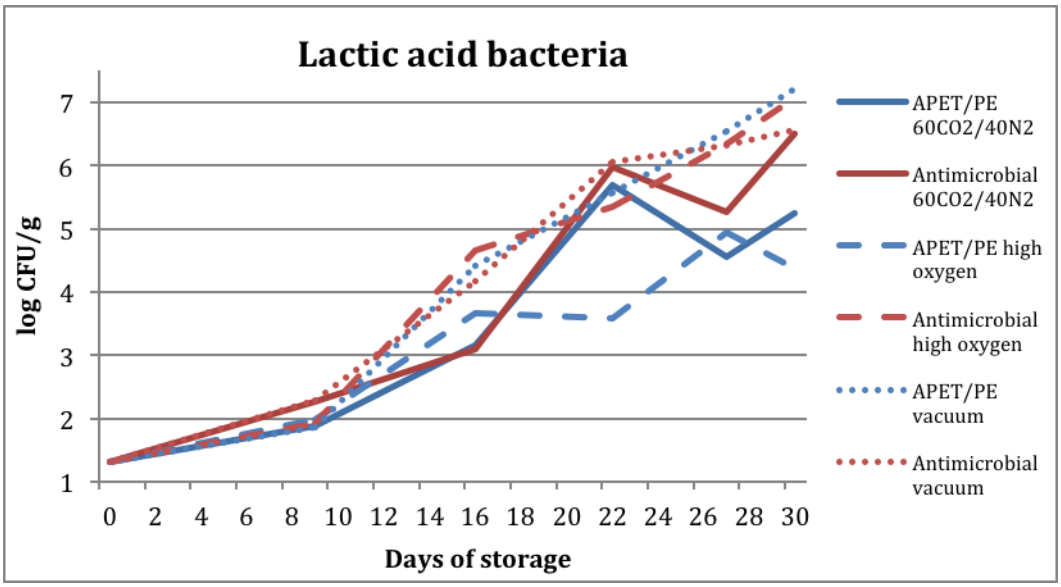


Figure 5.10 The changes in growth of *lactic acid bacteria* (log CFU/g) on beef loin steaks during 30 days of storage at 4 °C. The growth of *Lactic acid bacteria* was detected by the use of MRS-agar incubated at 25 °C for 5 days. The **red** lines show the growth on meat in antimicrobial packaging and the **blue** lines shows the growth on meat in APET/PE packaging. Dotted line: vacuum, dashed line: high oxygen (75% O_2/25% CO_2), and solid line: MAP (60% CO_2/40% N_2).

This graph, Figure 5.10, illustrate that the growth of *lactic acid bacteria* has had an overall increase in growth on all the samples during the storage period, (whit a quiet equal amount of growth). The high oxygen samples in APET/PE packaging material had slowest growth. The remaining samples had a similar growth of *lactic acid bacteria*, except the samples packed in APET/PE material with a modified atmosphere of 60% CO_2 and 40% N_2. The growth of *lactic acid bacteria* on these samples has been slower in the end of the storage period; decreased slightly after 22 days and then increased again after 27 days.

The vacuum packaged samples, and the samples with antimicrobial packing material and high oxygen atmosphere, had a steady (and the largest growth of *lactic acid bacteria*).
The meat samples packed in modified atmosphere, 60% CO_2 and 40% N_2, had a similar growth of *Lactic acid bacteria* on both varieties, but the one whit antimicrobial packaging material had slightly higher increase in growth in the end of the storage period, while the samples with the APET/PE packaging material decreased.

***Brochothirx thermosphacta* detection:**

There was no growth of *Brochothirx thermosphacta* on any of the samples throughout the storage period.

5.5 pH

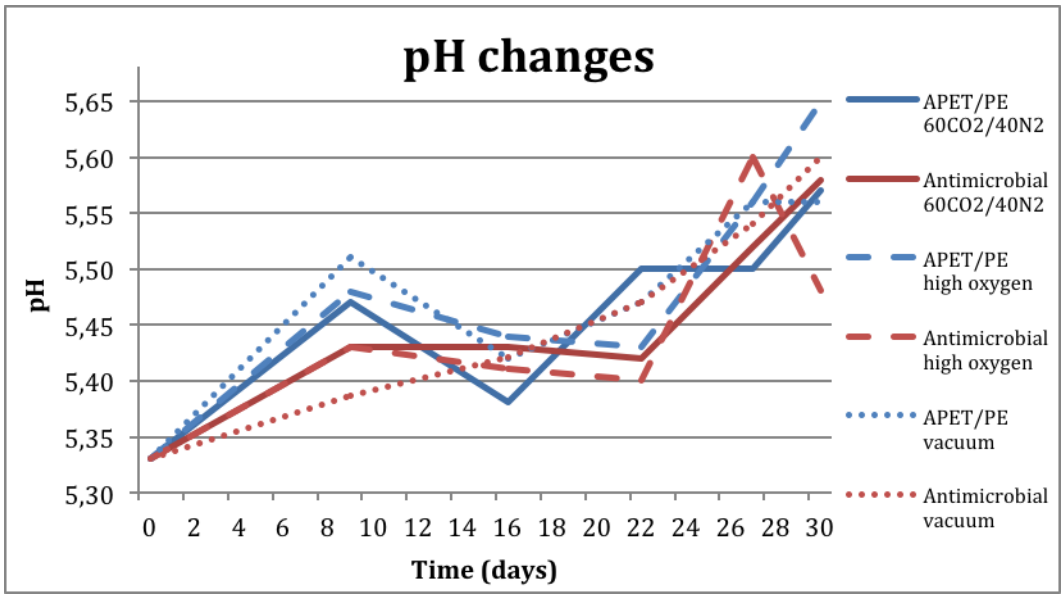


Figure 5.11 The changes in pH on beef loin steaks during 30 days of storage at 4 °C. The **red** lines show the concentration in antimicrobial packaging and the **blue** lines shows the concentrations in APET/PE packaging. Dotted lines illustrate the content in high oxygen samples. The solid lines illustrate packages packed with a gas mixture of 60% CO_2 and 40% N_2.

Most samples had a slightly increase of their pH-value with some fluctuations, Figure 5.11. Whit a start pH of 5,3 and up to about 5,6 at the end of the storage period. The samples in high oxygen with antimicrobial packaging material had also a drop in the end of the storage period, day 27 to day 30. The samples in vacuum with antimicrobial packaging material were the only variants that had a steady increase in pH.

5.6 Statistical analysis

General Linear model

The parallel was included as one of the factors at the first run of General Linear model (GLM) in Minitab. This was done to exclude greater differences between the parallel than between the different samples. No p-values lower than 0, 05 was found, which indicates that it was no significantly differences between the parallels. The second time, the GLM was run without parallels, which gave these results: The packaging material did not have any significant effect on drip loss, total count or growth of *lactic acid bacteria* alone. But packaging material combined with gas mixture gave a significant difference in drip loss, total count and *lactic acid bacteria* between the different samples. The growth of *lactic acid bacteria*, day, and day multiplied with day gave also significantly differences. This is also the only factor that had significantly effect on pH.

Packaging material, and packaging material multiplied with day gave a significantly difference in L*-,a*-, and b*- values, between different samples. The gas mixture gave also a significant difference in a*- and b*-values.

Appendix, table 1

One-way ANOVA

Appendix, table 2 *MAP = $60CO_2/40N_2$

6. Discussion

6.1 Gas concentrations

One would expect a reduction of the O_2 content, in the headspace of the packages during the storage period. The decrease of O_2 is due to the bacterial metabolism, because some microorganisms consume oxygen (Tortora et al. 2007). The results shown in Figure 5.1 indicate that the O_2 content of the packages with high oxygen remained fairly constant, but with a slight decrease towards the end of the storage period for the antimicrobial packages. It was a small reduction of the O_2-content for packages with CO_2/N_2, during the storage period. There were also a minimal increase of the O_2 content in the antimicrobial packages with CO_2/N_2 in the beginning of the storage period, but after 9 days the O_2 content in these was also reduced. There were minimal differences in oxygen content between the two packaging materials combined with high oxygen atmosphere and CO_2/N_2. This suggests that they both have almost the same, excellent oxygen transmission. But, the APET/PE laminate has a higher oxygen transmission rate (OTR) according to table 3.1, materials and methods, which may explain why high oxygen packs with APET/PE packaging has higher oxygen content than the antimicrobial packaging.

The CO_2 content in the packages was as expected, due to both original gas compositions, figure 5.2. In the packages with CO_2/N_2 there was a clear reduction of the CO_2 content until after 9 days of storage. From the 9th day, the CO_2 content in these packages was relatively constant. This was as expected, because CO_2 will be dissolved in meat, the meat consumes CO_2 and will eventually become saturated. The CO_2 content will therefore not be further reduced and it will remain in the packages at a relatively similar level. In the packages with high oxygen it was only a minimal reduction in CO_2 level, followed by a slightly increase at the end of the storage period. The CO_2 level in the antimicrobial packages was slightly higher in both atmospheres, versus APET/PE packages.

The results was as expected and the differences in CO_2 and O_2 content between the two packaging materials are minimal. Which. This will have a limited impact on the color changes, pH, drip loss, and bacterial growth in meat, within the same atmosphere. These results are in accordance to the findings by (Pettersen & Hansen 2012), a publication in modified atmosphere packaging of meat, where the exact gas composition was applied.

6.2 Color changes

One cannot expect major differences in the color CIELAB values (L*, a*, b*) in the two packaging materials. But regarding the packaging methods, will give greater differences. The results for L*-, a*- and b*-values, figure 5-3-5.5, indicates just that, but in addition to variations between the meat with different packing methods there were also variations between the parallels. It was initially large color variations on the meat samples used in this research, from relatively dark to a lighter red color. This may explain why the differences between the parallels/replicates were greater than it normally would be if the color differences at the time of packaging were miner.

The L* value tell us the "lightness" of the meat, the higher the L* value is, the brighter is the meat color and vice versa. It is expected that the L* value should be higher for meat packed in high oxygen versus meat stored in the absence of O_2, as vacuum packaging. High O_2 concentration causes a temporary bright red color on meat; oxygen binds to the muscle pigment myoglobin, forming oxymyoglobin, which will gradually be oxidized to metmyoglobin and cause a grey/green/brown color on the meat (figure 3.2) (Nollet et al. 2012). Towards the end of the storage period, some of the meat samples was discolored, or had grey stains. These samples were not chosen for Minolta, other samples were picked out instead. Figure 5.3 corresponds to this theory, the results shows that the L* value was higher in high oxygen meat versus meat in CO_2/N_2 and vacuum, this applies for both materials.

According to One-way ANOVA (appendix table 2a), at day 9 the meat in high oxygen with antimicrobial packaging had a significantly higher L* value than meat in vacuum with antimicrobial and APET/PE packaging. For day 16 it was almost the same, but it had no longer a significantly higher L * value than meat in vacuum with antimicrobial packaging. At day 16 meat in CO_2/N_2 with APET/PE had a significantly higher L* value than meat in vacuum with APET/PE. At day 22 the meat samples in high oxygen with antimicrobial and

with APET/PE packaging had a significantly higher L * value than meat in CO_2/N_2 and in vacuum with APET/PE. At day 27 there were no significant differences in L * values between the samples, and at day 30, meat in high oxygen with APET/PE had a significantly higher L * value than the meat samples in CO_2/N_2 with antimicrobial and the meat in vacuum with APET/PE.

The redness of the meat (a* value) were affected by the packaging method, and less affected by the packing materials, figure 5.4. Meat in high oxygen with antimicrobial packaging for instance, showed higher proportion redness, compared with the meat in high oxygen with APET/PE packaging. This also applies for meat in vacuum and CO_2/N_2.
The a* value were reduced for the meat with antimicrobial packaging and increased for on the meat with APET/PE packaging after 27 days of storage.

According to One-way ANOVA (appendix table 2b), at day 9 the meat samples in high oxygen with antimicrobial packaging had a significantly higher a* value than the remaining variants. The meat in CO_2/N_2 with antimicrobial packaging had a significantly higher a * value than meat packed with APET/PE. The meat samples in vacuum with APET/PE- and antimicrobial packaging, had a significantly higher a * value than meat packed in CO_2/N_2 with APET/PE. At day 16 the meat in vacuum and in high oxygen with antimicrobial packaging had a significantly higher a* value than the meat packed in CO_2/N_2 with antimicrobial and with APET/PE, and a higher value than meat in high oxygen with APET/PE. At day 22 the meat samples in high oxygen with antimicrobial packaging had a significantly higher a * value in relation to the remaining variants, except the meat samples in vacuum with antimicrobial packaging. By day 27 the meat with antimicrobial packaging had a significantly higher a * value than the meat in CO_2/N_2 with APET/PE. And at day 30 there were no significant differences.

The yellowness of the meat (b* value), is expected to be greater in high oxygen meat compared to vacuum packed meat and meat stored in CO_2/N_2. The b* values illustrated in figure 5.5 indicates this. Since meat stored in absence of O_2, as vacuum packaging,

deoxymyoglobin – the reduced form of myoglobin (Fe^{2+}) results in purple color, and therefore will have less yellowness (figure 3.2). The packaging method has had a greater effect on the b* value, compared to the packaging material. But the packaging material had also a small effect on the b* value, since meat in the antimicrobial packaging had a higher b* values compared to the meat in APET/PE packaging, especially for the meat with high oxygen atmosphere.

According to One-way ANOVA (appendix table 2c) at day 9, 16 and 22 the meat samples in high oxygen with antimicrobial packaging had a significantly higher b * value than the remaining variants. At day 9 the meat in CO_2/N_2 with antimicrobial packaging and high oxygen with APET/PE had a significantly higher b * value than the meat in CO_2/N_2 with APET/PE. At day 22 the meat in high oxygen with APET/PE had a significantly higher b * value than the remaining variants, except the meat in high oxygen with antimicrobial packaging, which had a higher L* value. And the meat in vacuum with antimicrobial packaging had a significantly higher b * value than the meat in CO_2/N_2 and in vacuum. At day 27 the meat in high oxygen with antimicrobial packaging had a significantly higher b * value than the remaining variants, except for the meat in high oxygen with APET/PE. At day 30 the meat in high oxygen with APET/PE had a significantly higher b * value than the remaining samples.

The results are in accordance to the finding by (Pettersen et al. 2013) (Li et al. 2012) and (Insausti et al. 1999). Where the L*-, a*- and b*-value was highest on meat stored in high oxygen atmosphere and modified atmosphere with CO_2, compared to vacuum-packed meat.

6.3 Drip loss

The illustrated drip loss in figure 5.6 shows, for most of the samples, a constant trend of a slight increase during the storage period. As expected the drip loss was greater for the vacuum-packed meat compared to the meat packed in modified atmosphere. The increased drip loss in vacuum packaged meat may partly be caused by the fact that the meat was pressed or squeezed during packaging. It seems that the packaging material also had some effect on the drip loss because the APET/PE packaging material has caused some greater drip loss on the meat compared to the antimicrobial packaging. There is one exception; meat in APET/PE that was packed in high oxygen had a higher water loss than meat in the antimicrobial packaging.

The generally greater drip loss of meat packed with APET/PE packaging may be partially due to that the antimicrobial bottom film is slightly thinner and less bendable compared to APET/PE bottom film as shown in table 3.1, materials and methods. This sometimes causes that small liquid amount drains out when opening the packaging, especially for the vacuum packed meat. The drip loss was in general slightly lower for all the samples at 9 days of storage, compared to the other sample points. At the sample points the packaging were usually weighed immediately after removing the meat, but at the first sample point this was done in the end of the day. It cannot be excluded that some of the liquid may have dried/evaporated before weighing.

According to One-way ANOVA (appendix table 2d) at day 9 and 16 the meat in vacuum with APET/PE packing had a significantly higher drip loss than the remaining variants, except the meat in vacuum with antimicrobial packaging. The meat in vacuum with antimicrobial packaging and in CO_2/N_2 with APET/PE had a significantly higher drip loss than the meat packaged in high oxygen with APET/PE. At day 16 these samples had also a significant higher drip loss than the meat in CO_2/N_2 with antimicrobial packaging. At day 22 the meat samples in high oxygen and vacuum, with antimicrobial packaging and the meat in vacuum with APET/PE had a significantly higher drip loss than the meat samples in high oxygen with APET/PE. At day 27, the meat in vacuum with APET/PE and antimicrobial packaging had a significantly higher drip loss than the meat in high oxygen with APET/PE and antimicrobial packaging. At day 30 the meat samples in high oxygen and vacuum with APET/PE and

antimicrobial packaging had a significantly higher drip loss than the meat in CO_2/N_2 with APET/PE.

The results are in accordance to the finding by (Pettersen & Hansen 2012) and (Pettersen et al. 2013), where the same packaging methods and modified atmospheres were used. Vacuum packaging meat had higher drip loss than meat in CO_2/N_2 atmosphere, and meat in CO_2/N_2 atmosphere had higher drip loss that meat stored in high oxygen. The drip loss also increased more during the storage period in these publications, which are in contrast with results from this study.

6.4 pH

It was expected that the pH of meat packed with CO_2/N_2 should be slightly lower compared to the meat packed in vacuum and high oxygen. Since CO_2 will dissolve in the product and "react" with water, which may cause presence of H^+- ions – and become acid and cause a lower pH. But this storage study did not show any sign of this, figure 5.7. The results reveal no clear difference in pH between the six varieties, after day 9. The measured values were somewhat variable. This might be because the samples were taken straight out of the refrigerator, and had not managed to reach room temperature before the measuring of pH. Room temperate samples could have provided more stable test results.

According to One-way ANOVA (appendix table 2e) there were no significantly differences in pH between the samples before day 22. At day 22, the meat in CO_2/N_2 with APET/PE had a significantly higher pH than the meat in high oxygen with antimicrobial packaging. At day 27, the meat in high oxygen with antimicrobial packaging had a significantly higher pH than the meat in CO_2/N_2 with APET/PE. At day 30, the meat in high oxygen with APET/PE had a significantly higher pH than the meat in high oxygen with antimicrobial packaging.

The results are in contrast to findings reported by (Hur et al. 2013), where the meat had a decrease in pH over time. pH values of meat packed in 30% CO_2/ 70% N_2 was slightly higher compared to the meat packed in vacuum. This was correct in the first two weeks of the storage period. But after 14 days, the pH of meat stored in vacuum was higher than pH of meat stored in CO_2/N_2.

6.5 Microbial growth

6.5.1 Total bacterial growth

The results of total bacteria numbers shown in Figure 5.7, was as expected to have greatest growth in the vacuum-packed meat, especially in the end of the storage period. In contrast results, the two packaging materials did not give quite the desired results. Meat with antimicrobial film, with CO_2/N_2 or vacuum, had a greater total number of bacteria throughout the storage period than meat with APET/PE-packaging. This applies especially to vacuum-packed meat. The total number of bacteria in meat with high oxygen on the other hand, has been lower for meat with antimicrobial packaging compared to the meat packed with APET/PE. This combination of gas and material has resulted in the lowest total bacterial growth on the meat surface. This may indicate that high oxygen in combination with antimicrobial film provides the best efficacy to inhibit aerobic bacteria on meat. However, because contact between material and product is required to inhibit the total bacterial growth and since it is in general expected greater growth on meat stored in oxygen compared with meat with CO_2/N_2 atmosphere, this cannot be concluded (Appendini & Hotchkiss 2002), (Tortora et al. 2007).

According to One-way ANOVA (appendix figure 2f) there were no significant differences in the total bacterial number between the different combinations of packaging material and gas mixture, before after 27 days of storage. After 27 days of storage, vacuum packed meat with both packaging materials were significantly different from meat with high oxygen and antimicrobial packaging, with a higher total bacterial number. This applies to the last sampling point, but additionally the meat in CO_2/N_2 with antimicrobial packaging were also significant higher in total bacterial number from meat in high oxygen with the same packaging material.

These results are in accordance to the findings by (Soldatou et al. 2009) and (Nissen et al. 1996), even though different gas compositions were used in these experiments. Vacuum and 70%CO_2/30% N_2 for the first one, and vacuum and 50%CO_2/50% O_2 atmosphere for the second publication.

6.5.2 Enterobacteriaceae

Figure 5.8 illustrates the growth of *Enterobacteriaceae* in the different package methods. The figure shows clearly that the lowest growth of *Enterobacteriaceae* is found on the meat packed in high oxygen with APET/PE- or antimicrobial packaging, with no distinct differences in between.

The greatest growth of *Enterobacteriaceae* was found on the vacuum-packed meat, with APET/PE- and antimicrobial packaging material. The low growth rate of *Enterobacteriaceae* in high oxygen meat was as expected. Although Enterobacteriaceae are facultative anaerobic organisms, which means that they can grow equally well with, or in absence of O_2, they grow more rarely in aerobic conditions. It is not until after 22 days of storage the growth of *Enterobacteriaceae* increased substantially from <1 to 4/5 log CFU/g. The growth of Enterobacteriaceae on vacuum packed meat packaged with antimicrobial film was reduced after 27 days of storage. A lower log value was detected for the last sampling.

The results from the last two samplings gave some uncertain values, which may explain this decline. Because the growth of Enterobacteriaceae was very low and stable at the time for the first three sampling points, the same dilutions were used for the fourth sampling point. Suddenly it occurred a rapid increase in growth of *Enterobacteriaceae* on meat samples packed in vacuum. It was used too low dilution of the samples at the fourth sampling point. This resulted in a large number of bacterial colonies, that grew close together on the VRBGA-medium, which made them difficult to count. Only three out of four parallels for APET/PE vacuum samples was possible to count. By splitting and counting 1/4 or 1/8 of the colonies on the agar plates, and then multiply the counted number by the number of splitting they could be counted. All the parallels for antimicrobial vacuum samples were possible to count by the same method. Log values for *Enterobacteriaceae* on vacuum samples was detected, but with a certain inaccuracy.

Because it was used to high dilutions on the samples with vacuum packaged meat at the first conduction, in the end of the storage period. That resulted in minor growth of colonies, which again led to uncertain values. And the detection on growth of *Enterobacteriaceae* in vacuum-packed meat was completed twice. By the second detection on the growth of *Enterobacteriacea*, it was only used two parallels of each vacuum variant with APET/PE and antimicrobial, because it was not possible to obtain enough samples from all four parallels.

Two parallels of each variant had coagulated and microbial samples could therefore not be obtained through the pipette tip.

According to One-way ANOVA (appendix, figure 2g) there were no significant differences in the growth of *Enterobacteriaceae* between the variants at the first three sample points. However, at the last two sample points, after 27 and 30 days, the vacuum packed meat in APET/PE- and antimicrobial packaging were significantly different from the remaining variants, with a higher growth of *Enterobacteriaceae*.

These results are in accordance to the findings by (Soldatou et al. 2009) and (Berruga et al. 2005)N, with highest growth of *Enterobacteriaceae* in vacuum packaged meat compared to meat in a modified atmosphere.

6.5.3 Pseudomonas

It is usually expected to be some growth of *Pseudomonas* on meat with high oxygen atmosphere. Figure 5.9 shows the growth of bacteria that was detected on CFC-agar, which should be selective for *Pseudomonas*. But it was not detected any oxidase positive tests on these colonies. This analysis indicated no growth of *Pseudomonas* on the meat samples throughout the storage period.

6.5.4 Lactic acid bacteria

Figure 5.10 illustrates the growth of *lactic acid bacteria* (LAB), and shows that the differences between the package variants were small. The greatest growth of LAB can be expected in meat packaged with the absence of O_2 like vacuum packaging, and least growth on high oxygen-packaged meat. The meat packaged in high oxygen with APET/PE packaging material is the variant with the lowest growth of LAB. The meat in high oxygen with antimicrobial packaging had a similar growth of LAB as the vacuum packed meat, which had the absolute greatest growth of LAB.

According to One-way ANOVA (appendix figure 2i), no significant differences in the growth of LAB in the two first, and the penultimate sample point was found. At the third sample point, the meat packaged in vacuum and CO_2/N_2 with antimicrobial packaging was significantly different with a higher growth of LAB in relation in high oxygen meat with

APET/PE packaging. At the last sample point, meat in vacuum CO_2/N_2, and high oxygen with antimicrobial packaging, and vacuum packed meat with APET/PE packaging was significantly different from the high oxygen meat with APET/PE packing, and had a higher growth of LAB.

Different effects of modified atmosphere packaging on bacterial growth have been reported (Nissen et al. 1996) and (Berruga et al. 2005), which are in accordance with these results. Even though the oxygen content used in this work are slightly different (higher/lower); the highest growth of LAB is found on vacuum packed meat, compared with meat stored in MAP. According to the findings by (Soldatou et al. 2009) when it comes to growth of LAB, the findings in this work have some differences, with a higher growth of LAB on meat stored in MAP, compared with vacuum. This might be caused by the use of a higher concentration of CO_2, which may contribute to a higher growth of LAB.

The growth of TVC and LAB are expected to be nearly equal, or actually greater for TVC, since LAB usually is a part of the TVC. The findings by (Viana et al. 2005) and (Berruga et al. 2005) confirms this theory, with a higher proportion and TVC vs. LAB. In this case, there have been a lower total number of bacteria, compared to the growth of LAB. This suggest that the PCA-media has failed to adapt all the *lactic acid bacteria* growing, which i the predominant spoilage flora

Bacterial growth vs. pH, color and drip loss.
- Changes in pH and bacterial growth are often correlated, but in this study it is no clear connection.
- There is a clear connection between fluid loss and bacterial growth, because there are greatest fluid loss and bacterial growth in the vacuum-packed meat.
- There are no significant connection between bacterial growth and color.
- Packing method / gas atmosphere has greater significance for color changes.

7. Conclusion

The aim of this study was to investigate the impacts of a specific antimicrobial film in packaging of beef loin steaks, regarding to the quality development and shelf life during cold storage. Based on the color, drip loss, pH and microbial measurements ant the results. The conclusions from this study can be summarized as follow:

- Microbial growth
  - The results show that the antimicrobial packaging had no better inhibition of bacterial growth compared to the modified atmosphere packaging.
  - Modified atmosphere on the other hand, had a greater effect on the inhibition of bacterial growth. High oxygen packaging had a lower total number of bacteria, and practically no growth of Enterobacteriaceae, compared to the meat packaged in CO_2/N_2 and vacuum. The growth of Enterobacteriaceae was also relatively low in the meat packed with CO_2/N_2, and a high total bacterial numbers does not necessarily conclude that the meat is of lower quality.
  - The storage stability of the vacuum packed meat was relatively for 22-25 days, and MAP consisting of 60 % CO_2/ 40% N_2 about 30 days. When it comes to the storage capability of MAP, high oxygen consisting of 75 % O_2/ 25 % CO_2, it was also about 30 days. But the bacterial growth was unexpected, extremely low. This seems strange considering that meat stored in high oxygen, usually turns bad long before meat packed in CO_2.
- Color, drip loss and pH
  - The values for color, liquid loss, and the pH of the meat in the two package materials were nearly identical, but the a* value (redness) was higher, and the fluid loss was slightly lower for the meat in antimicrobial packaging.

- The main conclusion
  - Modified atmosphere packaging with CO_2/N_2 in combination with APET/PE packaging had the best effect in increasing the quality and durability, considering that high oxygen eventually will result in a rancid flavor of the meat. While the antimicrobial packaging had no generating effect of inhibiting bacterial growth, but resulted in lower drip loss and higher a * - and b * values in relation to the meat with APET/PE packaging.
  - The reason for the differences in color values and the loss of fluid, which has seemed to be somewhat different in the two packaging materials might due to the fact that the material that was used consisted of two different assemblages of polymers. If this research was done again, not necessarily with this particular antimicrobial film, it may be appropriate to use an identical film without the antimicrobial components for comparison.

8. Suggestions for further studies

- Investigate/ study growth of other types of spoilage bacteria

- Use different storage conditions; different temperature and time horizons.

- Performing the experiment on other types of meat and marine products

- One could test the effects of other types antimicrobial material

- Perform a new preparation of the antimicrobial material, before any new optionally test experiment of the effect are carried out. Perhaps there is a need for higher concentration of the active component, do give positive results

- The use of a film that has one, or more, antimicrobial components in addition to silver ions, may give better results?

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Appendix

Table 1. General Linear model in Minitab. The factors that caused significant differences between the samples, when it comes to the response; Drip loss, the L*-,a*- and b*-value, Total number, *Enterobacteriaceae Pseudomonas spp.*, *Lactic acid bacteria*, and pH

Analysis of variance for		Low P-value
L*-value	Packing material	0,012
	Packing material x day	0,010
a*-value	Packaging material	0,000
	Gas mixture	0,025
	Packing material x day	0,000
b*-value	Packaging material	0,000
	Gas mixture	0,001
	Packing material x day	0,000
Drip loss	Packing material x Gas mixture	0,002
Total number	Packing material x Gas mixture	0,039
Lactic acid bacteria	Day	0,001
	Packing material x day	0,029
	Day x day	0,046
pH	Day	0,000
	Day x day	0,000

Excluded the results for O_2 and CO_2, since theses are not essential for the meat qu

Table 2. One-way ANOVA in Minitab
The different responses versus packaging material and gas mixture

Table 2a) L*-value vs. packaging material and gas mixture

	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	47,88 ^{b}	44,24 ^{ab}	40,01 ^{a}	43,82 ^{ab}	43,15 ^{ab}	39,33 ^{a}
16	45,62 ^{b}	43,36 ^{ab}	39,06 ^{a}	43,88 ^{ab}	44,36 ^{b}	41,42 ^{ab}
22	46,41 ^{b}	42,3 ^{ab}	42,99 ^{ab}	46,55 ^{b}	41,53 ^{a}	41,17 ^{a}
27	44,74 ^{a}	43,45 ^{a}	40,58 ^{a}	44,1 ^{a}	43,93^{a}	40,62 ^{a}
30	43,78 ^{ab}	41,77 ^{a}	42,19 ^{ab}	47,77 ^{b}	43,32 ^{ab}	39,95 ^{a}

Table 2b) a*-value vs. packaging material and gas mixture

	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	17,94 ^{d}	15,15 ^{c}	14,45 ^{bc}	11,64 ^{ab}	9,09 ^{a}	12,59 ^{b}
16	16,1 ^{b}	10,57 ^{a}	15,31 ^{b}	10,83 ^{a}	10,16 ^{a}	12,74 ^{ab}
22	19,01 ^{b}	12,97 ^{a}	14,62 ^{ab}	13,65 ^{a}	10,95 ^{a}	12,02 ^{a}
27	15,46 ^{b}	14,68 ^{b}	15,05 ^{b}	12,31 ^{ab}	9,5 ^{a}	12,37 ^{ab}
30	9,35 ^{a}	10,82 ^{a}	13,17 ^{a}	16,2 ^{a}	15,12 ^{a}	14,79 ^{a}

Table 2c) b*-value vs. packaging material and gas mixture

	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	8,76 ^{c}	4,3 ^{b}	2,19 ^{ab}	3,8 ^{b}	0,83 ^{a}	1,75 ^{ab}
16	6,86 ^{b}	1,2 ^{a}	2,26 ^{a}	4,06 ^{a}	2,17 ^{a}	1,52 ^{a}
22	9,11 ^{d}	2,05 ^{ab}	3,1 ^{b}	5,65 ^{c}	1,18 ^{a}	1,46 ^{a}
27	7,25 ^{b}	3,36 ^{a}	1,94 ^{a}	4,14 ^{ab}	1,55 ^{a}	1,33 ^{a}
30	3,62 ^{a}	2,21 ^{a}	2,11 ^{a}	7,92 ^{b}	3 ^{a}	2,18 ^{a}

Table 2d) Drip loss vs. packaging material and gas mixture

	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	4,72 ^{ab}	4,36 ^{ab}	5,94 ^{bc}	3,64 ^{a}	5,45 ^{b}	7,68 ^{c}
16	5,17 ^{ab}	4,62 ^{a}	7,17 ^{bc}	4,65 ^{a}	6,1 ^{b}	8,43 ^{c}
22	6,21 ^{b}	5,12 ^{ab}	7,2 ^{b}	3,33 ^{a}	5,68 ^{ab}	8,18 ^{b}
27	5,51 ^{a}	5,93 ^{ab}	7,73 ^{b}	5,46 ^{a}	6,56 ^{ab}	8,52 ^{b}
30	6,79 ^{b}	6,55 ^{ab}	6,98 ^{b}	6,71 ^{b}	5,5 ^{a}	7,67 ^{b}

Table 2e) Total number vs. packaging material and gas mixture

	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	2 [a]	2,64 [a]	2,37 [a]	2,46 [a]	2,27 [a]	2,08 [a]
16	2,53 [a]	2,65 [a]	4,13 [a]	3,6 [a]	3,24 [a]	2,63 [a]
22	2,82 [a]	4,93 [a]	5,18 [a]	2,91 [a]	4,46 [a]	3,62 [a]
27	3,03 [a]	4,23 [ab]	5,58 [b]	3,51 [ab]	3,16 [ab]	5,67 [b]
30	2,27 [a]	5,29 [b]	5,94 [b]	3,86 [ab]	4,14 [ab]	5,83 [b]

Table 2f) *Enterobacteriaceae* vs. packaging material and gas mixture

	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	1 [a]	1 [a]	1 [a]	1 [a]	1 [a]	1 [a]
16	1 [a]	1,15 [a]	1 [a]	1 [a]	1 [a]	1,8 [a]
22	1 [a]	2,21 [a]	1 [a]	1 [a]	2,06[a]	1 [a]
27	1,43 [a]	1,65 [a]	4,63 [b]	1 [a]	1,31 [a]	4,35 [b]
30	1 [a]	1,64 [a]	3,58 [b]	1,25 [a]	1,57 [a]	4,63 [b]

Table 2g) *Pseudomonas* vs. packaging material and gas mixture

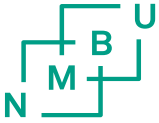
	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	1,3 [a]	1,3 [a]	2,07 [b]	1,3 [a]	1,3 [a]	1,3 [a]
16	1,3 [a]	1,3 [a]	2,11 [a]	1,3 [a]	1,3 [a]	1,81 [a]
22	1,3 [a]	2,09 [a]	2,63 [a]	1,42 [a]	3,13 [a]	2,17 [a]
27	1,3 [a]	1,92 [a]	4,67 [b]	1,92 [a]	1,65 [a]	4,92 [b]
30	1,78 [a]	1,85 [a]	4,24 [b]	1,5 [a]	2,21 [ab]	5,23 [b]

Table 2h) *Lactic acid bacteria* vs. packaging material and gas mixture

	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	1,92 [a]	2,26 [a]	2,27 [a]	1,96 [a]	1,87 [a]	1,85 [a]
16	4,64 [a]	3,1 [a]	4,15 [a]	3,65 [a]	3,15 [a]	4,4 [a]
22	5,34 [ab]	5,98 [b]	6,05 [b]	3,58 [a]	5,69 [ab]	5,56 [ab]
27	6,34 [a]	5,27 [a]	6,31 [a]	4,94 [a]	4,54 [a]	6,54 [a]
30	7,07 [b]	6,51 [b]	6,56 [b]	4,37 [a]	5,23 [ab]	7,21 [b]

Table 2i) pH vs. packaging material and gas mixture

	Antimicrobial packaging			APET/PE packaging		
	High oxygen	MAP	Vacuum	High oxygen	MAP	Vacuum
9	5,43[a]	5,43[a]	5,39[a]	5,48 [a]	5,47[a]	5,51[a]
16	5,41 [a]	5,43 [a]	5,42 [a]	5,44 [a]	5,38 [a]	5,42 [a]
22	5,40 [a]	5,42 [ab]	5,47 [ab]	5,43 [ab]	5,50 [b]	5,47 [ab]
27	5,60 [b]	5,52 [ab]	5,54 [ab]	5,56 [ab]	5,50 [a]	5,56 [ab]
30	5,48 [a]	5,58 [ab]	5,60 [ab]	5,65 [b]	5,57 [ab]	5,56 [ab]



Norwegian University
of Life Sciences

Postboks 5003
NO-1432 Ås, Norway
+47 67 23 00 00
www.nmbu.no