

# Packaging Methods and Storage Time

## Effects on Beef Quality

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## Packaging methods and storage time – Effects on beef quality

### Abstract

Consumers base their purchasing choices on perceived quality and a bright red colour of beef is to many a sign of freshness and good meat quality, making colour the most important quality attribute for retailers. The most common consumer packaging types for beef in Sweden is high-oxygen modified atmosphere packaging (MAP), with the gas composition (80% O<sub>2</sub>; 20% CO<sub>2</sub>). The high-oxygen content gives the beef a stable bright red oxymyoglobin colour that is desirable to consumers at the moment of purchase and the addition of 20–30% CO<sub>2</sub> prolongs the shelf life by inhibiting bacterial growth. Nonetheless, the high level of oxygen promotes oxidation of both proteins and lipids, giving an inferior product compared with packaging systems that exclude oxygen. Vacuum packaging expands the shelf life of beef even further than high-oxygen MAP and the tenderization continues throughout the storage time. However, the purple deoxymyoglobin colour and the visible purge loss in the vacuum bag are thought to be unattractive to consumers. The aim of this thesis was to study how storage time and packaging methods affects the quality of beef, comparing high-oxygen MAP with vacuum packaged and/or skin packed beef, with emphasis on colour, Warner–Bratzler shear force, sensory quality and oxidation of proteins.

In conclusion, high-oxygen MAP systems for beef steaks were found to negatively influence shear force and water-holding capacity as well as the sensory attributes tenderness, meat flavour and juiciness, compared with beef steaks packaged in skin pack or vacuum. High-oxygen MAP might also lead to decreased  $\alpha$ -tocopherol values and increased oxidation of proteins. Moreover, no clear differences was found between skin packed and vacuum packed steaks for shear force, sensory quality and total loss, however, skin packed steaks had lower purge loss which might be more appealing to the consumers in retail display. Most research is done on frozen meat due to practical reasons. As the consumers mostly eat meat fresh, the differences in quality that comes with freezing should be taken into consideration when planning research.

*Keywords:* Beef, high-oxygen modified atmosphere packaging, vacuum package, skin pack, shear force, sensory quality, colour, frozen beef.

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# Dedication

Till min älskade underbara familj...

*Ett spontant tal tar tre veckor att förbereda.*

Winston Churchill

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## List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

- I Lagerstedt, Å., Enfält, L., Johansson, L., Lundström, K. (2008). Effect of freezing on sensory quality, shear force and water loss in beef *M. longissimus dorsi*. *Meat Science*, 80, 457-461.
- II Lindahl, G., Lagerstedt Å., Ertbjerg, P., Sampels S., Lundström, K. (2010). Ageing of large cuts of beef loin in vacuum or high oxygen modified atmosphere – Effect on shear force, calpain activity, desmin degradation and protein oxidation. *Meat Science*, 85, 160-166.
- III Lagerstedt, Å., Lundström, K., Lindahl, G. (2011). Influence of vacuum or high-oxygen modified atmosphere packaging quality of beef *M. longissimus dorsi* steaks after different ageing times. *Meat Science*, 87, 101-106.
- IV Lagerstedt, Å., Ahnström, M. L., Lundström, K. (2011). Vacuum skin pack of beef – a consumer friendly alternative. *Meat Science*, 88, 391-396.

Papers I-IV are reproduced with the permission of the publisher.

The contribution of Åsa Lagerstedt Norström to the papers included in this thesis was as follows:

- I Participated in the planning of the experimental work together with supervisors. Participated in the collection of samples and performed the laboratory work. Participated in the evaluation of the results and was responsible for writing the manuscript.
- II Participated in the planning of the experimental work together with supervisors. Participated in the collection of samples. Prepared the samples for calpain and desmin analysis. Performed the shear force analysis, colour and water holding capacity measurements. Participated in the evaluation of the results and the writing of the manuscript.
- III Participated in the planning of the experimental work together with supervisors. Participated in the collection of samples and performed the laboratory work. Participated in the evaluation of the results and was responsible for writing the manuscript.
- IV Participated in the planning of the experimental work together with supervisors. Participated in the collection of samples and performed the laboratory work. Participated in the evaluation of the results and was responsible for writing the manuscript.

## Abbreviations

ATP	adenosine triphosphate
CO <sub>2</sub>	carbon dioxide
DeoxyMb	deoxymyoglobin
IMF	intramuscular fat
LD	<i>M. Longissimus dorsi</i> , the beef loin
LMS	least squares means
MA	modified atmosphere
MAP	modified atmosphere packaging
MetMb	metmyoglobin
MHC	myosin heavy chain
MLC	myosin light chain
NADH	nicotinamide adenine dinucleotide
O <sub>2</sub>	oxygen
OxyMb	oxymyoglobin
PMB	premature browning
PVC	polyvinyl chloride
SE	standard error
TBARS	thiobarbituric acid reactive substances
WB	Warner-Bratzler
WHC	water holding capacity
WOF	warmed-over flavour



# Introduction

Beef quality can be different things depending on who you ask. For consumers it is important that beef looks nice and that it tastes good to eat. Other important factors when consumers buy meat are price, degree of marbling, origin and packaging. Retailers want carcasses with a low amount of fat and meat that has a high colour stability and water holding capacity. In this project beef that has been packaged in modified atmosphere with high-oxygen content was compared with vacuum packaged and skin packed beef with the focus on colour, shear force and sensory quality. Meat colour depends on both the degree of pigment and the muscle structure. Retailers have reported an increasing problem with colour stability in beef. If meat is packaged in modified atmosphere with high oxygen content the colour stability of beef increases, but other quality parameters such as shear force, tenderness, juiciness and meat flavour are negatively affected due to oxidation of proteins and fat. The aim of this project is to optimize beef treatment after slaughter.

In Sweden, the self sufficiency rate for beef and veal in 2009 was approximately 61%. The beef produced comes to a large part from young bulls and cows of dairy breeds (Swedish Board of Agriculture, 2010). Bulls tend to grow faster than steers and heifers and they produce leaner carcasses. However, meat from bulls tends to be tougher and more inconsistent in tenderness compared with meats from steers and heifers (Zhang *et al.*, 2010; Jeremiah *et al.*, 1991). This variation is due to a natural diversity in the animal material and to quality differences between sexes.

## Meat quality

From one point of view meat tenderness is a simple characteristic, the meat is either tender or tough. However, the explanation for the variations in meat tenderness is far from simple because many different internal and external factors are involved. To understand what meat quality is and how it develops, one must first understand some fundamental aspects about muscle structure.

### Muscle structure

The muscle cell is among the most highly organized cells in the animal body (Lonergan & Lonergan, 2008). Muscle tissue in a living animal consists of about 75% water and 20% protein. The remaining 5% is mainly fat with small amounts of minerals and glycogen. The percentage of intra muscular fat varies considerably among species and individuals, and among muscles (Hocquette *et al.*, 2010). Skeletal muscle is composed of muscle fibres, where each fibre is a single cell with associated connective tissue. The muscle fibres are bound together into bundles and each muscle fibre consists of bundles of myofibrils surrounded by the plasma membrane, sarcolemma. The myofibrils are surrounded by a fluid phase, the sarcoplasm (Lawrie & Ledward, 2006). The basic structure of skeletal muscle fibre can be seen in Figure 1.

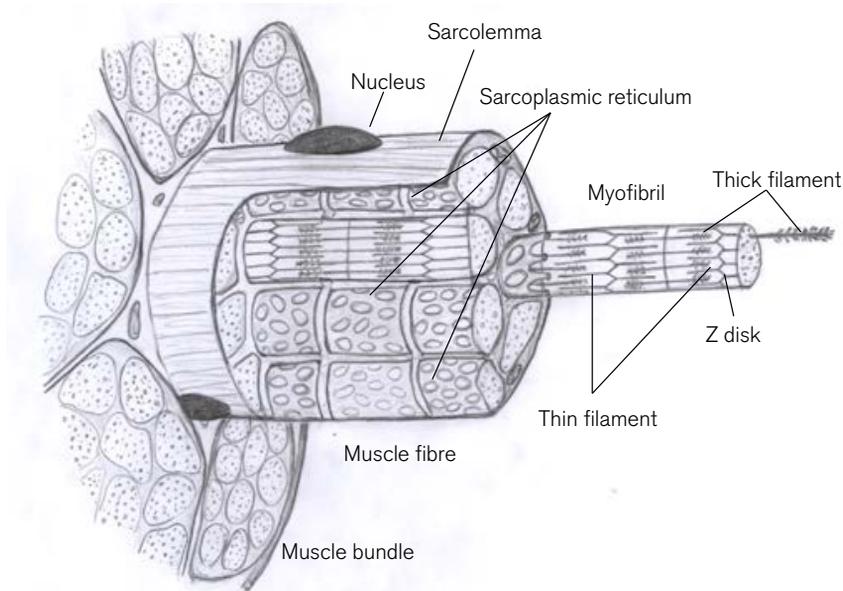


Figure 1. The structure of skeletal muscle fibre, modified after Silverthorn *et al.*, (2001).

The different muscle proteins have a wide range of physiological functions in the muscle. Most muscle proteins are myofibrillar proteins (~60%) and the others are sarcoplasmic proteins (~30%), such as enzymes and myoglobin, and structural proteins (~10%) such as the connective tissue proteins collagen and elastin (Lawrie & Ledward, 2006). When looking at a muscle cell under a microscope, a distinct banding pattern can be observed. This appearance is due to specialized organelles, myofibrils, found in muscle cells. Myofibrils are the contractile “machinery” of the cell, and can be divided into thin and thick filaments consisting of actin and myosin, respectively. The protein actin is a small globular molecule (G-actin) that is aggregated end to end to form the twisted double strand (F-actin) of the globular units that is the thin filament (Lawrie & Ledward, 2006). The contractile protein myosin is a hexamere consisting of two heavy chains and two pairs of myosin light-chains (Figure 2). The myosin molecule consists of two regions, a head and a tail. The tail of myosin forms the backbone of the thick filament and the globular head region extends from the thick filament and interacts with actin in the thin filament (Lonergan & Lonergan, 2008; Moss *et al.*, 1995).

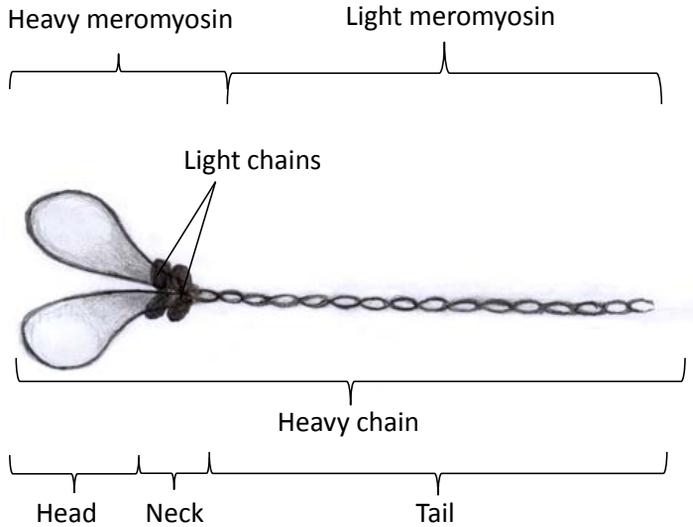


Figure 2. Schematic picture of a myosin molecule.

When the muscle contracts, the thick and thin filaments interact via the head region of myosin forming actomyosin. In living muscle during contraction, the ATPase activity of myosin provides energy for myosin bound to actin to rotate and ultimately pull the thin filaments toward the centre of the sarcomere (Lonergan & Lonergan, 2008). This shortens the myofibril, the muscle cell and eventually the muscle leading to contraction. The myosin and actin can disassociate when a new molecule of ATP is bound to the myosin head (Silverthorn *et al.*, 2001). The banding patterns arise from alternating, protein dense A-bands and less dense I-bands within the myofibril. The less dense I-band is made up primarily of thin filaments whereas the A-band is made up of thick filaments and some overlapping thin filaments (Silverthorn *et al.*, 2001). Bisecting the I-bands are dark lines known as Z-lines (Huxley & Hanson, 1954) and the area between two Z-lines is called a sarcomere (Figure 3). The sarcomere repeats approximately every 2.4  $\mu\text{m}$  (when at rest) along the myofibril axis (Lawrie & Ledward, 2006), thus the banding pattern seen in the microscope. Each sarcomere contains all the structural elements needed to perform the physical art of contraction at molecular level (Huff-Lonergan *et al.*, 2010).

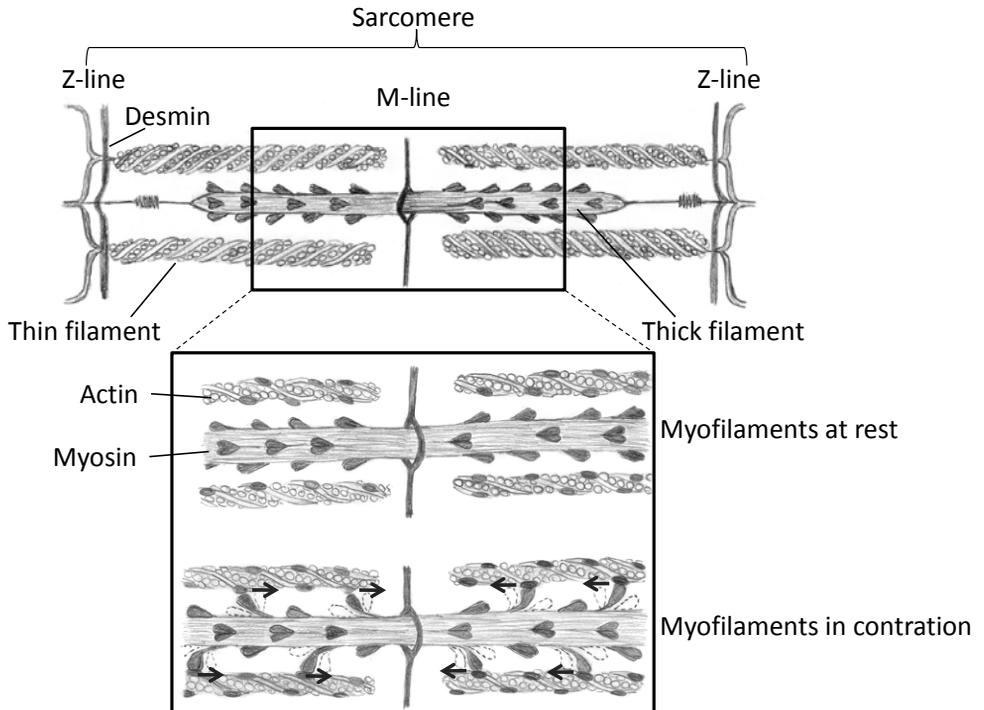


Figure 3. The structure of a sarcomere during muscle contraction.

### Muscle becomes meat

The transformation from living muscle to meat starts after exsanguination as the carcass temperature declines and the energy of the cell is low. In the living muscle the blood oxygenates the muscles. When the oxygen supply is depleted the cells' energy metabolism changes from the aerobic to the anaerobic pathway of adenosine triphosphate (ATP) production (Thompson *et al.*, 2006). Generating ATP through the anaerobic pathway gives less ATP and results in the end product, lactic acid, being accumulated in the muscle, thus lowering the pH. The rate and extent of the pH fall is important for the meat quality. When the ATP level is too low to maintain the relaxed state of the muscles the carcass goes into *rigor mortis*, each muscle fibre individually gradually increasing muscle stiffness (Huff-Lonergan & Lonergan, 2005; Honikel *et al.*, 1983). This increase in muscle stiffness is generally significant when pH has declined to about 6 (Thompson *et al.*, 2006). At *rigor mortis* the overlapping actin and myosin filaments attach irreversibly and form cross-bridges between each other. These rigor bonds

are the origin of the stiffness that develops in *postmortem* muscle. To get tender meat it is important to have as stretched muscles and as long sarcomeres as possible to minimize these rigor bonds.

Meat tenderness is determined by the amount and solubility of connective tissue, sarcomere length, rate of proteolysis during ageing and *postmortem* metabolism (Warner *et al.*, 2010; Koohmaraie & Geesink, 2006), whereas the tenderization process is mainly the result of proteolytic enzymes present in the muscle. The tenderizing process in itself is affected by several factors such as the age, sex, breed and diet, as well as the handling of the slaughter animal pre-slaughter. After slaughter pH, temperature, suspension method, rate of glycolysis and location of and the physiology of the cut and packaging method all affect the ultimate tenderness of meat. Cooking time, method and temperature, and the final inner temperature of the meat also considerably affect the final eating experience (Yancey *et al.*, 2011).

### Tenderization

During *postmortem* ageing, proteolytic enzymes degrade the proteins and loosen up the structure of the myofibrils (Koohmaraie & Geesink, 2006). This can be seen by an increased Myofibrillar Fragmentation Index or degradation of specific proteins such as the cytoskeletal protein desmin (Therkildsen *et al.*, 2002). The effect of ageing depends on the time and temperature during ageing and also on the growth rate of the animal up to slaughter, because a fast growth rate increases the activity of proteolytic enzymes in meat (Therkildsen *et al.*, 2002). The tenderization process is mainly the result of the activity of proteolytic enzymes present in the muscle. The calpain system is by many considered to be responsible for most of the *postmortem* tenderization (Koohmaraie & Geesink, 2006). The calpain system is composed of several isoforms of the proteolytic enzyme calpain, and an endogenous inhibitor of the calpains, calpastatin (Lonergan & Lonergan, 2008; Goll *et al.*, 2003). The two best-characterized isoforms are  $\mu$ -calpain and m-calpain. Both  $\mu$ - and m-calpain are cysteine proteases that require the sulphhydryl group in the active site to be reduced to have proteolytic activity. Substrates for calpains are proteins that form the cytoskeletal framework of the muscle cell are among others desmin and talin (Bilak *et al.*, 1998). The large proteins of the thick and thin filaments such as titin, nebulin, and troponin-T play a key role in muscle cell integrity (Huff-Lonergan *et al.*, 2010). Disruption of these proteins, especially titin, vinculin, nebulin and desmin, could result in myofibril fragmentation and loss of

muscle cell integrity, and leading to tenderization of the muscle (Huff-Lonergan *et al.*, 2010; Taylor *et al.*, 1995).

## Protein oxidation

Oxidation is the main cause of quality deterioration during processing and storage of meat (Xiong, 2000). Whereas the oxidation of lipids has been studied in depth, the oxidation of meat proteins has yet to be thoroughly studied (Lund *et al.*, 2011) to understand how proteins in *postmortem* muscle are affected after exposure to oxidative stress (Xiong, 2000). The depletion of endogenous antioxidants makes the postmortem muscle more susceptible to oxidation (Xiong, 2000). This was shown in beef during the ageing process in which the sulfhydryl content gradually decreased while the protein carbonyl content increased (Martinaud *et al.*, 1997). Most initial studies of protein oxidation has so far been limited to examining the content of protein carbonyls (Lund *et al.*, 2011). High-oxygen content in beef packaging head space promotes oxidative changes in the meat, leading to tougher meat (Kim *et al.*, 2010; Clausen *et al.*, 2009; Grobbel *et al.*, 2008; Zakrys *et al.*, 2008). Why meat gets tougher when packaged in high-oxygen MAP has not yet been fully clarified, but different theories have been suggested and research in the area is ongoing. The main two theories are that i) the enzymes involved in the tenderisation process might be oxidized by the high-oxygen content leading to a slower or interrupted tenderisation process (Rowe *et al.*, 2004) and that ii) intermolecular cross-links involving disulfide bonds are formed in myosin leading to a tougher meat (Kim *et al.*, 2010; Lund *et al.*, 2008; Lund *et al.*, 2007).

## Colour

Consumers base their purchase choices on the perceived meat quality (Troy & Kerry, 2010). Colour becomes synonymous with red meat quality (Renner & Labas, 1987) and is the major selling point since the average consumer uses discolouration as an indicator of lack of freshness and nutritional value of meat. A high colour stability of fresh meat is essential for retailers. It has been shown that the willingness of consumers to purchase beef steaks was correlated to visual appearance scores going from red to purple to brown (Carpenter *et al.*, 2001).

The colour of meat is determined mainly by the concentration and chemical state of the heme pigments. The sarcoplasmic protein mainly responsible for the red colour of meat is myoglobin; however, other related proteins containing a heme group such as haemoglobin and cytochrome C also affect beef colour (Mancini & Hunt, 2005). The amount of myoglobin depends on species, breed, sex, age and type of muscle (Lawrie & Ledward, 2006). Myoglobin consists of a single polypeptide protein globin and a prosthetic group heme that consists of a porphyrine ring with a centrally located iron atom that has six coordination sites (Mancini & Hunt, 2005; Jeyamkondan *et al.*, 2000). Four of the six coordination sites are bound to pyrrole nitrogen on the porphyrine rings (Mancini & Hunt, 2005; Horton *et al.*, 2002). Of the last two sites, one on each side of the heme plane, position five is connected to the nitrogen of histidine on the globin protein and the sixth is available to bind different ligands (Mancini & Hunt, 2005; Jeyamkondan *et al.*, 2000). The colour differences in fresh beef depend on which ligand is attached to this sixth site, deciding the chemical status of myoglobin. Deoxymyoglobin (DeoxyMb) is the reduced form of myoglobin ( $\text{Fe}^{2+}$ ) which gives a purple colour when oxygen is absent (Figure 4). This form can be found when the meat is just cut or is packaged in vacuum. The bright red oxymyoglobin (OxyMb) is that reduced ( $\text{Fe}^{2+}$ ) pigment form where  $\text{O}_2$  occupies the ligand position. The meat is oxygenated, so called blooming. Metmyoglobin (MetMb) is the oxidized form of myoglobin ( $\text{Fe}^{3+}$ ) and results in a brown or greenish colour. When oxygen is present there is an oxidation of OxyMb or DeoxyMb to the brown MetMb (Renerre, 1990). However, an increased oxygen concentration causes a significant decrease in the rate of metmyoglobin formation (Ordóñez & Ledward, 1977).

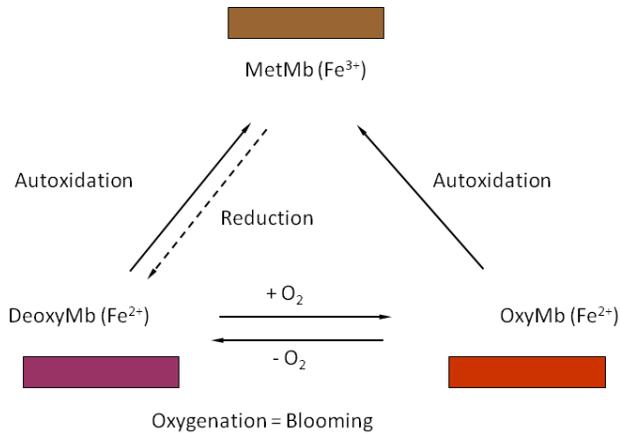


Figure 4. The colour cycle of myoglobin in meat. Figure by G. Lindahl.

While there is still enzymatic reducing activity in the meat, MetMb will be transformed to the purple DeoxyMb, which can be transformed again to the bright red OxyMb. As the exposure to oxygen increases, the OxyMb layer penetrates deeper beneath the surface of the meat. The depth of the OxyMb layer depends on the meat's temperature, oxygen partial pressure, pH and the competition for oxygen by other respiratory processes (Mancini & Hunt, 2005). Meat packaged in modified atmosphere with high oxygen content will develop a thicker deeper penetrating layer of OxyMb with increasing storage time (Seyfert *et al.*, 2004). In a beef steak packaged in high-oxygen modified atmosphere the OxyMb layer can penetrate the whole steak. Colour stability, i.e. how stable the DeoxyMb or OxyMb is before discolouration appears, can be measured as amount of surface area covered by metmyoglobin (MetMb) and depends partly on the same factors that determine the oxygen penetration, as well as microbial growth and the meat's reducing activity (Mancini & Hunt, 2005). Animal variation due to genetics also influences colour stability (Mancini & Hunt, 2005). Discolouration is due to the amount of MetMb that is located between the OxyMb on the surface and the interior DeoxyMb. When meat starts to get discoloured the layer of MetMb gradually thickens and moves towards the meat's surface (Mancini & Hunt, 2005). An important factor for meat colour is the MetMb reducing activity of metmyoglobin-reducing system, which reduces MetMb to DeoxyMb and then back to OxyMb, using the

NADH pool in the muscle. However, this is only possible until the storage of NADH is depleted with progressing ageing time. Metmyoglobin reductase activity has been found in both pork (Mikkelsen *et al.*, 1999) and beef (Madhavi & Carpenter, 1993). Muscles with low colour stability such as *M. Psoas major* had higher oxygen consumption and lower metmyoglobin reductase activity compared with *M. Longissimus dorsi* (Madhavi & Carpenter, 1993).

### Premature browning

The phenomenon of premature browning (PMB) can be described as the development of well done appearance within the interior of the meat when temperature ensuring safety has not been reached (Clausen *et al.*, 2009; King & Whyte, 2006; Seyfert *et al.*, 2004; Tørngren, 2003; Hunt *et al.*, 1999; Hague *et al.*, 1994). The phenomenon is directly influenced by the chemical status of the myoglobin at the time of cooking and occurs for meat with predominantly bright red OxyMb and brown MetMb colour (Hunt *et al.*, 1999). Both OxyMb and MetMb are more sensitive to heat denaturation compared with purple DeoxyMb and will therefore turn brown at a lower endpoint temperature when cooked (King & Whyte, 2006; Hunt *et al.*, 1999). The colour of cooked meat is therefore a poor indicator of a microbiologically safe inner temperature. This is especially important for minced meat that has been packaged in high-oxygen modified atmosphere (MAP) and that has a bright red OxyMb colour in the package. Even though it is mostly studied on minced meat, beef steaks also can show PMB. In a study where beef steaks exposed to 50 or 80% oxygen, steaks looked well done even if the internal temperature only reached 62°C (Clausen, 2004). Therefore, using a thermometer instead of judging the colour visually has been promoted (King & Whyte, 2006; Seyfert *et al.*, 2004), this is especially important for minced meat.

### Marbling

Intramuscular fat, or adipose tissue, deposited between the muscle bundles is called marbling, and is visible to the human eye as spots of fat. The amount of marbling varies among animal species, breeds, muscle type, gender, age and feeding intensity and is linked to number and size of adipocytes (Hocquette *et al.*, 2010). Ruminants' fat tissue contains a higher proportion

of saturated and lower proportions of polyunsaturated fatty acids compared to monogastric species due to the saturation of fatty acids from the feed by the bacteria in the rumen (Wood & Enser, 1997). Grass fed ruminants, however, have high levels of n-3 fatty acids (Fredriksson Eriksson & Pickova, 2007; Wood & Enser, 1997). An increased level of polyunsaturated fatty acids in the muscle can increase the susceptibility to lipid oxidation (Högberg *et al.*, 2002). If the dietary intake of vitamin E is increased discolouration and lipid oxidation can be delayed in beef (Faustman *et al.*, 1998).

The accretion rate of marbling depends on the rate of muscle growth; heavily muscled animals with a high glycolytic activity show less marbling and with increasing muscularity fat will also be diluted (Hocquette *et al.*, 2010). This may be why bulls generally have less pronounced intramuscular fat compared with heifers and steers (Wierbicki *et al.*, 1956). Marbling is regarded as a quality criterion of beef in many countries and is judged positively in Asia and North America, whereas an excess of marbling is mostly unpopular in European countries (Hocquette *et al.*, 2010). The amount of marbling contributes mainly to experience of flavour and juiciness and indirectly influences meat tenderness (Hocquette *et al.*, 2010; Webb & O'Neill, 2008; Jeremiah *et al.*, 2003). At the moment of purchase, consumers prefer steaks with low marbling, but when tasting the meat they actually prefer steaks with higher degree of marbling (Jeremiah *et al.*, 1992). Low levels of marbling may lead to dry and less tasty meat (Hocquette *et al.*, 2010). A reduction of the fat content therefore may adversely affect the eating satisfaction of meat (Webb & O'Neill, 2008). The fat content and the possibly negative effect of red meat on consumers' cholesterol levels have become a major health concern (Resurreccion, 2003; Verbeke *et al.*, 1999). The increased consumption of processed meat products shows changes in consumer taste and preferences (Grunert, 2006), products that often have a higher degree of fat compared to the lean meat.

## Water-holding capacity

One of the most important quality aspects of meat is its water-holding capacity (WHC). It can be defined as the ability for meat to retain its own water or take up added water during application of any force (Offer & Knight, 1988). Any system prolonging shelf-life of beef will be subjected to purge (Troy & Kerry, 2010). Purge affects the economic traits for the

industry, loss of yield in fresh meat and processed products, as well as decreased palatability for the consumer. Weight loss due to purge can average as much as 1-3% in fresh retail cuts (Offer & Knight, 1988) and unacceptable WHC costs the meat industry millions of dollars annually (Huff-Lonergan & Lonergan, 2005).

Water in the muscle fibre serves as a lubricant, as well as a medium to transport metabolites (Puolanne & Halonen, 2010). Most water in muscle is held within the structure of the muscle and muscle cells. Specifically, within the muscle cell, water is found within and between the myofibrils and between the myofibrils and sarcolemma (Offer & Cousins, 1992; Offer & Trinick, 1983). Approximately 85% of the water in the muscle cell is held in the myofibrils (Huff-Lonergan & Lonergan, 2005). During the conversion of muscle to meat, pH decreases until it has approached the isoelectric point of actin and myosin where they have no electrical charge and tend to lose the water normally bound to them. As the pH value declines, the net charge of the proteins that make the myofibril approaches zero and the repulsion of structures within the myofibril is reduced, allowing the myofibrillar proteins to pack more closely together leaving less room for water (Figure 5). When pH is declining proteins also tends to denature, which also effects the WHC of the muscle (Huff-Lonergan & Lonergan, 2005).

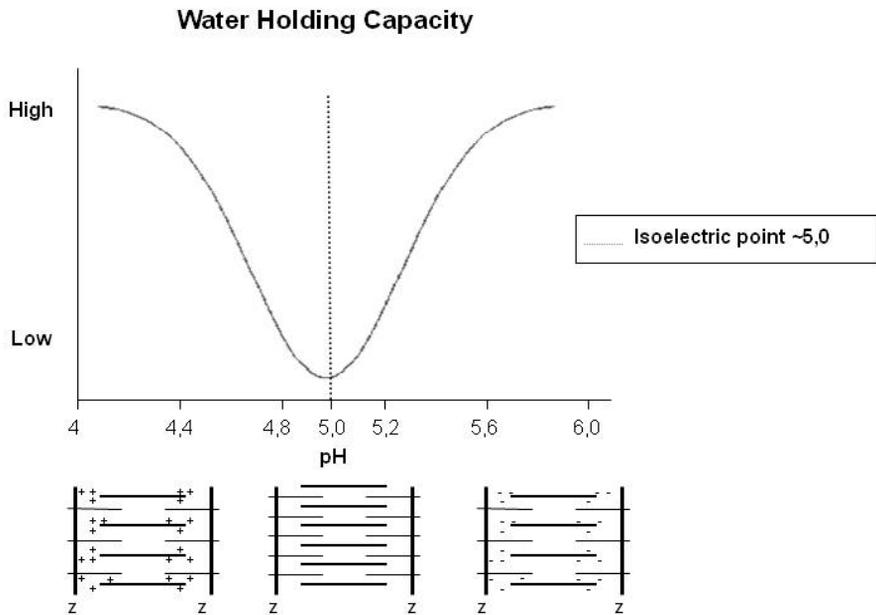


Figure 5. Water holding capacity around the isoelectric point, with the charges around the Z-discs reaching zero around pH 5.0, allowing the myofibril structure to pack more closely together, leaving less space for water.

Several factors influence WHC such as pH decline, ionic strength and oxidation (Huff-Lonergan & Lonergan, 2005). Temperature during storage also affects WHC, a high temperature giving more purge. Sample size also affects WHC, a thin slice gives higher purge compared with a larger cut (Huff-Lonergan, 2009). Lateral shrinkage of the myofibrils occurs during rigor. This shrinkage can be transmitted to the entire muscle cell if proteins that link myofibrils together and myofibrils to the cell membrane (such as desmin) are not degraded (Huff-Lonergan & Lonergan, 2005). Limited degradation of cytoskeletal proteins may lead to increased shrinkage of the overall muscle cell, which is ultimately translated into drip loss. Oxidation causing limited degradation of cytoskeletal proteins may lead to increased shrinkage of the overall muscle cell, and therefore lower WHC (Huff-Lonergan & Lonergan, 2005). Packaging may also affect WHC because modified atmosphere packaging (MAP) has higher purge compared with vacuum packed meat (Taylor *et al.*, 1990). WHC has been shown to

increase with ageing of meat due to structural changes both chemically and physically through structural breakdown, creating a “sponge effect” which disrupts the moisture loss channels and physically entraps free water (Farouk *et al.*, 2010).

## Freezing of meat

Freezing induces ice-crystal formation in the muscle, which will cause leakage of fluids when the meat is thawed (Risvik, 1995). Freezing also damages the cell membranes (Rahelić *et al.*, 1985) which leads to a lower water holding capacity and a higher cooking loss (Wheeler *et al.*, 1990) and consequently a risk of less juicy meat. It is known that freezing beef also affects tenderness. Frozen samples from LD had lower shear force values compared to chilled meat aged the same time (Shanks *et al.*, 2002) and beef tenderized for 7 days and then frozen and thawed had the same shear force values as chilled meat tenderized for 21 days (Enfält *et al.*, 2004). The most favourable way of freezing meat is fast freezing at low temperature (Dransfield, 1994), which is possible at industry level. The activity of calpain is stopped when meat is frozen, but the enzymes are not destroyed, and the activity is resumed after thawing (Dransfield, 1994). If the meat is frozen prior to ageing, the tenderization process needs to be completed after thawing to get properly aged meat (Dransfield, 1994). However, it is preferable to age meat prior to freezing since a long ageing time may be required after thawing and most meat that is sold frozen is cooked without further ageing (Dransfield, 1994).

It is presumed that it is the intracellular ice formation, disrupting the cell during freezing, that leads to the decreased peak force in frozen and thawed meat (Shanks *et al.*, 2002). The most cellular damage of freezing of beef is obtained when freezing the meat at  $-22^{\circ}\text{C}$  since large ice crystals both intra- and intercellular were formed (Rahelić *et al.*, 1985). The damage in the cellular structure of meat that has been frozen has been reported to encourage oxidation and increase the level of oxidation products (Campo *et al.*, 2006), which may lead to increased amount of off-flavours.

## Packaging

For all meats the two main costs for production is animal husbandry and storage (Smulders *et al.*, 2006). The high investment in production and storage makes it even more important to have the right type of packaging, also considering environmental damage and recycling of packaging material. The role of meat packages is to protect the meat and increase the shelf life but it should also help to sell the product. Many interrelated factors influence the shelf life and freshness of meat such as temperature, oxygen, endogenous enzymes, moisture, light and most important microorganisms (Zhou *et al.*, 2010). Consumers have become increasingly concerned about food-borne risks and personal health (Van Wezemael *et al.*, 2010). As the demand for food safety is increasing, cutting and packing of raw meat in air-permeable overwrap in individual stores are being replaced by case-ready centralized packaged meat. The meat packaging industry has therefore grown in many countries. In 2006, case-ready packaging of meat at the European market was 43% (Belcher, 2006).

### Modified atmosphere packaging

The most common consumer packaging method of beef in Sweden and most of the Western World is high-oxygen modified atmosphere packaging (MAP), with the gas composition 80% O<sub>2</sub> and 20% CO<sub>2</sub>. Packaging of fresh beef in high-oxygen MAP is predominantly used for retail display of steaks and minced meat. By using modified atmosphere packaging the shelf life of fresh red meat can be extended (Zhao *et al.*, 1994), compared with meat wrapped in air-permeable overwrap. The amount of meat that is packaged in high-oxygen MAP is increasing due to the increased colour stability and better hygienic quality of the meat compared with meat wrapped in air-permeable plastic (Jakobsen & Bertelsen, 2000). The high-oxygen content gives the beef a stable bright red oxymyoglobin colour that is desirable to consumers at the moment of purchase (Carpenter *et al.*, 2001). The addition of 20–30% CO<sub>2</sub> prolongs the shelf life by inhibiting bacterial growth (McMillin, 2008). However, high-oxygen MAP will allow growth of aerobic bacteria and therefore has a reduced shelf life compared to vacuum packaging (Jeremiah, 2001).

The disadvantage of a high-oxygen content when packaging beef in modified atmosphere (MA) is the increased level of lipid oxidation (Jakobsen & Bertelsen, 2000). It has also been shown that a high-oxygen

content in MAP can negatively affect the tenderness in beef (Clausen *et al.*, 2009; Clausen, 2004; Sørheim *et al.*, 2004; Tørngren, 2003) and increase cross-linking of proteins in pork (Lund *et al.*, 2007) and beef (Lund *et al.*, 2008). High-oxygen MAP has been shown to increase the breaking strength of individual beef muscle fibres (Lund *et al.*, 2008). Moreover, sensory attributes such as juiciness and meat flavour are negatively affected and the amount of off-flavour increases in high-oxygen MAP (Clausen, 2004; Sørheim *et al.*, 2004).

### Vacuum pack

Vacuum packaging extends the shelf life of beef even further than high-oxygen MAP and the tenderization continues throughout the storage time. Vacuum packing eliminates the air surrounding the meat and consequently the meat colour changes from a red oxymyoglobin colour to a purple deoxymyoglobin colour (Shay & Egan, 1987). However, the purple deoxymyoglobin colour of meat in vacuum package and the visible purge loss in the vacuum bag is thought to be unattractive to consumers, which is why vacuum packing is not as frequently used for retail display as MAP in Sweden. Purge loss may be susceptible to bacterial growth and this could be a problem when it occurs in vacuum bags. The most common use of vacuum packages in the meat industry is for ageing of wholesale meat. However, some retailers sell larger pieces of beef packed in vacuum at Swedish supermarkets.

### Skin pack

Skin pack is a relatively new packaging method on the Swedish market. The meat is placed in trays and the upper packaging film is heated and shrinks tightly around the meat and adheres to the trays when vacuum is drawn. Skin packed meat has a shelf life of 15–22 days (Belcher, 2006). One advantage of skin pack is that it produces almost no wrinkles in the packaging in which purge loss may collect (Vázquez *et al.*, 2004) and it is supposed to give less purge loss in the package compared to vacuum packing. Steaks in skin pack had lower weight loss during storage compared with vacuum packed steaks after 20 days of storage (Clausen *et al.*, 2009). Lower purge loss gives less fluid that can be substrate for bacterial growth. Since the colour of skin packed beef is displayed in the DeoxyMb state, oxidation issues are minimized (Belcher, 2006).

The heating of the upper packaging film and the tight disposition is thought to be one of the reasons why skin pack exhibited a slower microbial growth and a longer shelf life compared with vacuum packages (Vázquez *et al.*, 2004), which suggests that skin pack is a more desirable packaging solution. If the beef is kept fresh in retail display the meat colour turns purple and there may be problems with consumer acceptability. Studies have shown that skin packed steaks were less tender than vacuum packed steaks (Clausen, 2004; Vázquez *et al.*, 2004).

## Tenderness and meat flavour

The palatability of meat is determined by the combination of tenderness, juiciness and meat flavour (Koochmaraie *et al.*, 2002). Of these, tenderness is the most important for consumers. It has been shown that consumers find it difficult to choose beef in the supermarket because they are unsure of its quality, especially the level of tenderness (Dransfield, 1994). To improve tenderness of beef cuts, meat is aged after slaughter prior to retail display. In Sweden, a minimum ageing time is set to 7 days for beef by the meat industry. Consumer preference surveys for beef usually rates tenderness as the most important criterion, when comparing sensory attributes. Tenderness is the attribute which tends to vary the most. Inconsistency in beef tenderness is one of the major factors affecting consumer satisfaction and has been identified as one of the major problems facing the beef industry (Destefanis *et al.*, 2008; Koochmaraie, 1994). It has been shown that consumers could distinguish between different categories of tenderness (Wheeler *et al.*, 2004; Boleman *et al.*, 1997) and were willing to pay a premium for improved tenderness (Boleman *et al.*, 1997). Tenderness of beef can be evaluated both instrumentally and with sensory analysis.

The development of meat flavour is a complex system. The flavour of raw meat is bland, slightly metallic and serum like (Troy & Kerry, 2010). There are hundreds of compounds in meat that contribute to flavour and aroma, many of which are altered during storage and cooking (Calkins & Hodgen, 2007). When meat is cooked a series of thermally induced complex reactions take place between the many different non-volatile compounds of the lean and fatty tissue (Calkins & Hodgen, 2007). Because flavour of cooked meat depends on water soluble components as well as lipids, the distinct flavour differences between species (pork, lamb and beef) is not surprising (Troy & Kerry, 2010). Off-flavour such as warmed-over flavour (WOF) and rancidity is affected by the lipid oxidation and correlates

well with analytical attributes such as thiobarbituric acid reactive substances (TBARS) (Campo *et al.*, 2006).

## Sensory analysis

The science of sensory analysis is young compared to traditional sciences such as physics and chemistry. Sensory analysis can be a very useful tool when studying eating quality of beef. It can be used in quality control and shelf life evaluation, such as the colour stability of raw meat and in product development (Lawless & Heymann, 1998). The approach used and the choice of test used depend on what is sought. Defining meat quality demands knowledge of not only the intended usage but also of the meat itself (Risvik, 1995). There are several international ISO standards concerning sensory analysis regulation and how to perform different tests to try and control different types of bias. When putting together a professional panel the panellists have to be screened and trained, which is a time consuming and expensive task. A well trained panel is also expensive to maintain and needs constant evaluation and training. Which training is necessary depends on the question being assessed. A selected and trained panel can be used as an instrumental tool in descriptive analysis, making the score for the trait in question on a linear scale.

Descriptive analysis can be used to characterize certain flavours or odours, and can be correlated to instrumental measurements and consumer acceptance. One of the most common ways is a trained descriptive attribute panel testing meat for detecting differences in traits such as tenderness, juiciness and flavour intensity. A complement to a trained panel is the use of consumer test. The affective or preference test describes the liking of a product. A consumer panel gives information regarding acceptability of meat products. The panellists are usually trained to perform similarly, but in most cases one can observe clear differences between their scores. This can be due to different sensitivity to the attribute in question or misunderstanding of the scale (Tomic *et al.*, 2007). There are computer programs that can be used when screening and checking the panellist performance through graphical plots and summary statistics, such as “Panel Check” ([www.panelcheck.com](http://www.panelcheck.com)) (Naes *et al.*, 1996). Another available computer program registering the panel’s results is the EyeQuestion Software from Logic8 (Version 3.6; [www.logic8.com](http://www.logic8.com)). EyeQuestion is marketed as an all-in-one software solution for sensory and consumer

research since it covers panel management, multi channel data collection and analysis of data and reporting tools.

There are many other types of tests apart from descriptive analysis and profiling. Discrimination tests include triangular, duo-trio, paired comparison and ranking tests. Each one of them answers the question “Is there a difference between these samples?” The paired comparison and the ranking test can also answer the question whether there is a difference between samples regarding a specific attribute, such as tenderness. Descriptive test with a sensory panel have been the most frequently used test in this thesis, however, paired comparison has also been used in a consumer test.



## Aims

The overall aim of this thesis was to obtain further knowledge about how beef that has been packaged in modified atmosphere (MAP) with high-oxygen content behaves in comparison with vacuum packaged beef with focus on colour, shear force and sensory quality. The goal with the project is to optimize the treatment of beef after slaughter.

The specific aims of the presented work were:

- I To determine how sensory quality, shear force and water loss differ between beef stored either chilled or frozen before cooking.
- II To investigate how beef quality was affected when storing large beef cuts (10 cm long) in either high-oxygen MAP or vacuum directly, or first vacuum and then MAP.
- III To determine how sensory quality, shear force, colour and water loss differ between beef steaks in MAP with the gas mixture 80% O<sub>2</sub> and 20% CO<sub>2</sub> and vacuum packaging.
- IV To investigate how beef quality was affected when storing steaks in high-oxygen MAP, skin pack or vacuum.



# Methods

## The Swedish slaughter procedure

All animals within each study were slaughtered the same day. After stunning and exsanguination, beef carcasses were de-hided and eviscerated. The carcasses are then electrically stimulated (80V, 30s) approximately 30 min after exsanguination. Carcasses are then suspended in the Achilles tendon and split along the spine. Carcasses are transferred to the chilling room and cut the following day (day 1 *postmortem*). pH was measured between the 10-11 rib with a Knick portable pH-meter equipped with a combination gel electrode (SE104, Knick, Berlin, Germany). Carcasses selected for the trials had a pH in LD below 5.6 at 24 h postmortem. During commercial cutting the fore- and hind-quarters of the carcass were separated between the 10th and 11th rib. The meat used in our trials was beef *M. Longissimus dorsi* (LD), cut from the 11th rib to the last lumbar vertebrae, from both sides of the carcasses.

## Animal material and muscle sampling

### *Paper I*

Eight young bulls of Swedish Holstein breed (age 13-17 months) came from one farm and had the same feed. The weight of the carcasses ranged from 290 to 312 kg and the EUROP classification varied from O- to O+ and with a EUROP fatness from 2+ to 3. Day 1 postmortem the carcasses were cut and the LD were packed in vacuum bags. Each LD muscle was cut into 4 samples (in total 8 samples per animal). Samples for shear force analysis were 7 cm long and caudally distributed from the partitioning site (between the 10th - 11th rib) with the order 2, 7 and 14 days of storage. The rest of

the muscle was used for sensory analysis and these samples were approximately 30 cm long. The samples were weighed and vacuum packed then stored at 4°C for 2, 7 or 14 days (day 0 being the day of slaughter) after which the samples were either heat treated or frozen at -20°C in a freezing room.

### *Paper II*

Ten young bulls of Swedish Holstein breed (age 14–17 months) came from one farm and had the same feeding regime, *ad libitum* access to silage and barley. The weight of the carcasses ranged from 288 to 315 kg and the EUROP classification varied from O- to O+ and with a EUROP fatness score from 2+ to 3+. LD muscles were excised at 1 day postmortem and stored at 4°C until packaging for the different treatments at 2 days postmortem. Ten different treatments were included in the study: no ageing, ageing solely in vacuum for 5, 15 or 25 days, ageing in high-oxygen MAP (80% O<sub>2</sub> + 20% CO<sub>2</sub>) for 5 or 10 days, and ageing in vacuum for 5 or 15 days followed by MAP for 5 or 10 days (Table 1). Samples were aged in darkness in a chilling room at 4°C. Each LD was divided into five 10-cm-long pieces, giving 10 pieces per animal. Treatments were distributed along the LD locations according to an extended Latin Square design with randomized order of location on the first animal and of animal number. Each treatment included ten samples of LD. Thus LD from all ten animals and all the five locations were represented in each ageing system, and each LD location was represented by two animals. After ageing, samples for biochemical analysis were frozen and stored at -80°C, whereas samples for measurements of shear force and cooking loss were analysed fresh.

Table 1. *Experimental design of the ageing systems: 10 cm long pieces of M. longissimus dorsi cut 2 days post mortem were not aged (=control sample) or aged at 4 °C in vacuum for 5, 15 or 25 days or in high oxygen modified atmosphere (MA) for 5 or 10 days or in vacuum for 5 or 15 days followed by MA for 5 or 10 days.*

Ageing system	Ageing time (days)		
	Vacuum	MAP	Total
V0M0 (control)	0	0	0
V0M5	0	5	5
V5M0	5	0	5
V0M10	0	10	10
V5M5	5	5	10
V15M0	15	0	15
V5M10	5	10	15
V15M5	15	5	20
V25M0	25	0	25
V15M10	15	10	25

### *Paper III*

Ten young bulls of Swedish Red and White breed (age 12-16 months) came from one farm. The carcass weight ranged from 293 to 327 kg and the EUROP classification varied from O to O+ with a EUROP fatness score from 2+ to 3+. LD muscles were removed the day after slaughter and stored at 4°C until assigned to different treatments at 3 days postmortem. Next 15 cm of LD (starting from rib 11) was used for shear force measurements and the next 15 cm for sensory analysis. LD was cut in 3-cm steaks, weighed and assigned to five different treatments: no storage, storage in vacuum for 5 or 15 days and storage in vacuum for 0 or 5 days followed by MAP (80% O<sub>2</sub> + 20% CO<sub>2</sub>) for 5 or 10 days (Table 2). Each steak that was packaged in MAP was put on plastic straws in the trays in order for the oxygen to be able to penetrate into the meat evenly from all sides. The replicates within treatment were taken on the same site on the two LD muscles. The samples were aged in darkness in a chilling room at 4°C. After ageing the samples were frozen at -20°C until analysed.

Table 2. The different treatments in the order they were located on the LD. Starting anterior with V0M0 and moving posterior to the last treatment V5M10 within each analysis (shear force and sensory analysis). Day 0 being 3 days after slaughter

Ageing system	Vacuum	MAP	Total
V0M0	0	0	0
V5M0	5	0	5
V0M5	0	5	5
V15M0	15	0	15
V5M10	5	10	15

#### *Paper IV*

The animals in the study were six young bulls of beef breed crosses, age 16–20 months from two farms. The whole LD muscles from the two sides were packed in vacuum and aged for 7 days at 2°C at the slaughter plant. Each LD was then unpacked and cut into 3.5-cm steaks, weighed and packed using the three different packaging methods, vacuum, vacuum skin pack and high-oxygen modified atmosphere packaging with the gas composition 80% O<sub>2</sub> and 20% CO<sub>2</sub>. The steaks were then transported to the lab and stored in their respective package at 4°C for additional 0, 7 or 14 days, i.e. in total 7, 14 and 21 days *postmortem*. Steaks frozen at day 7 *postmortem* were used as a control for all other treatments. At each storage time the samples were unpacked, wrapped in aluminium foil and placed in a plastic bag before being frozen at -20°C until further analysis.

## Specific Methodology

### Instrumental tenderness

Instrumental tenderness was measured using the Warner-Bratzler (WB) method (Honikel, 1998). The cooked meat was stored at 4°C until evaluated the next day. The samples were cut into 1-cm-thick slices and then into strips minimum 30 mm long with a 100-mm<sup>2</sup> (10 × 10 mm) cross-section area. The strips were cut parallel to the longitudinal orientation of the muscle fibres. Shear force was measured on a minimum of 12 strips from each sample with a Stable Micro Systems Texture Analyser HD 100 (Godalming, UK), equipped with a WB shear force blade with a rectangular shaped cutting area of 11 mm × 15 mm. The cutting blade was 1 mm thick and had the speed of 0.83 mm/s when cutting through the

strips. Shear force was analysed as peak force and total energy (area under the curve).

## Sensory analysis

### *Paper I*

The meat was wrapped in aluminium foil and heat treated in a conventional oven at 150°C, until a final internal temperature of 69°C. Samples were cooled at room temperature and stored at 4°C until the following day and then cut in 3-mm-thick slices. Sensory analyses were performed both by a sensory panel and by consumers. The sensory panel included 8 assessors trained during one session. The sensory attributes judged by the panellists were tenderness, juiciness and meat flavour. The panel assessed the attributes on a scale from very low (1) to very high (10) intensity. In the consumer test, 157 consumers at a local supermarket were asked to choose which sample they preferred (chilled or frozen) from the same animal. The meat was the same as in the panel test.

### *Paper III*

The samples were heat treated in vacuum bags for 55 min in a 72°C water bath, turned after 25 min, thereafter chilled in running cold tap water for 30 min and then stored at 4°C until the next day and then cut in 3-mm-thick slices. The panel consisted of 6 semi-trained panellists (two training sessions). The meat served to the panel had room temperature and was served in duplicates on three digit randomly coded plates. The attributes judged were tenderness, juiciness and meat flavour. A category scale with 17 structured steps from 1 to 9 (1, 1.5, 2, 2.5 etc.) was used where 1 was low intensity and 9 was high intensity. The average of the two replicates was used in the statistical analysis.

### *Paper IV*

The steaks were heat treated in plastic bags in a water bath until a core temperature of 72–73°C, cooled in running tap water for 30 min and then stored at 4°C until the following day. The samples were then cut into slices 3–4 mm thick and all edges were trimmed to give a more uniform appearance. The slices were packed in tin foil and sorted into two sessions so that replicates would not be tested in the same session. Sensory analysis was performed with a sensory panel, composed of 8 assessors, selected using screening tests and PanelCheck ([www.panelcheck.com](http://www.panelcheck.com)). The attributes

tested were tenderness, juiciness, acidity and meat flavour, assessed on a scale from very low (1) to very high (9) intensity. The precooked samples were then distributed to the panel members for home testing at room temperature and the results were recorded using EyeQuestion Software from Logic 8 Version 3.6 ([www.logic8.com](http://www.logic8.com)).

## Colour

### *Paper III*

The colour was measured, using a Minolta CM-2500d spectrophotometer (Minolta Co, Ltd, Osaka, Japan) with specular reflectance excluded, 8 mm diameter measuring aperture, illuminant D65, 10° Standard Observer and CIE colour scale (CIE, 1976). The measuring aperture was covered with a glass plate, and the instrument was calibrated against a white plate ( $L^* = 97.29$ ,  $a^* = -0.07$ ,  $b^* = 0.12$ ). The average of four measurements across the surface was used. The colour of the meat was measured through a plastic covering film. Colour stability was assessed by colour measurements after blooming for 1 h and 3 h and then daily during 5 days of display in air at 4°C. The instrument measures reflectance between 400 and 740 nm at 10-nm intervals, and Kubelka-Munk K/S values were calculated. The relative content of deoxymyoglobin (DeoxyMb) was estimated by the ratio  $(K/S_{474}) / (K/S_{525})$ , the relative content of oxymyoglobin (OxyMb) by the ratio  $(K/S_{610}) / (K/S_{525})$  and the relative content of metmyoglobin (MetMb) by the ratio  $(K/S_{572}) / (K/S_{525})$  (Mancini *et al.*, 2003; Hunt *et al.*, 1991). K/S ratios at wavelengths not given by the instrument (474, 525, 572 nm) were calculated using linear interpolation of the reflectance values. The K/S ratios decrease when the relative content of the corresponding myoglobin form increases and the K/S ratios were therefore transformed in the diagrams in order to get the right impression when looking at the curves. The K/S ratio of OxyMb was transformed to  $[1 - (K/S_{610}) / (K/S_{525})]$ , the K/S ratio of DeoxyMb was transformed to  $[1.5 - (K/S_{474}) / (K/S_{525})]$  and the K/S ratio of MetMb was transformed to  $[2 - (K/S_{572}) / (K/S_{525})]$ .

### *Paper IV*

The colour was measured, using a Minolta CM 600d spectrophotometer (Minolta Co, Ltd, Osaka, Japan) with specular reflectance excluded, 8 mm diameter measuring aperture, illuminant D65, 10° Standard Observer and CIE colour scale (CIE, 1976). The measuring aperture was covered with a glass plate, and the instrument was calibrated against a white plate ( $L^* =$

97,56;  $a^*=-0,16$ ;  $b^*=0,02$ ). The average of four measurements across the surface was used. The colour of the meat was measured through a plastic covering film. Colour for both raw and cooked meat was recorded as a mean value of four measurements, taken on one of the duplicate steaks used for Warner-Bratzler shear force. Measurements of the raw steaks were taken after 1 h of blooming under PVC wrapping through the covering film. Measurements of cooked steaks were taken the day after cooking on a cut cross-section area of the steaks.

## pH

pH was measured before the meat was cut and packaged with a Knick portable pH-meter equipped with a combination gel electrode (SE104, Knick, Berlin, Germany). Ultimate pH was between 5.33-5.60 for all animals included in the four papers.

## WHC

Water holding capacity was calculated as weight loss as percent of initial weight. Purge loss was calculated as sample weight before packaging minus sample weight before heat treatment in percent of sample weight before packaging. Freezing loss was calculated as sample weight before freezing minus sample weight after freezing in percent of sample weight before freezing. Cooking loss was calculated as sample weight after cooking minus sample weight before cooking in percent of sample weight before cooking (Paper I-IV).

### *Paper I*

Purge or thawing loss and cooking loss were calculated separately for samples used for sensory analysis, whereas only the combined water loss (sum of purge/thaw and cooking loss) was measured for samples used for shear force analysis.

### *Paper III*

Samples were not repacked before freezing and thus freezing loss also included purge. Because the same cooking method was used for shear force and sensory analysis, loss (purge or thaw, cooking and total loss) was calculated as the combined mean value within treatment for the 4 samples used for shear force and sensory analysis (2 replicates each).

## Chemical analysis

### *Vitamin E*

Vitamin E content was analysed as  $\alpha$ -tocopherol content by extracting the antioxidant from the minced meat and then using high performance liquid chromatography (HPLC) (Högberg *et al.*, 2002). Duplicate samples were prepared by homogenizing tissue (1 g) in 2 ml ethanol using an Ultra-Turrax (Janke & Kunkel, IKA Werke, Straufen, Germany). To saponify each sample, 1.2 ml of 20% ascorbic acid, 0.6 ml methanol and 1.2 ml 17.9 M potassium hydroxide were added. Samples were then agitated in a 75°C water bath for 20 min. Samples were cooled and the tocopherol was extracted twice using 4×2 ml hexane. The hexane was evaporated under nitrogen gas. The tocopherols were dissolved in the mobile phase and injected in the HPLC. The mobile phase was 95% acetonitrile:methanol (1:1 v/v) and 5% chloroform with a flow rate of 1.2 ml/min. Tocopherols were detected at the excitation wavelength of 290 nm and emission wavelength of 327 nm.

### *Calpain activity and desmin*

Frozen meat samples were finely chopped and each 2-g sample was homogenized using an Ultra-Turrax T25 Mixer at 13,500 rpm for 3×20 s in 10 volumes of cold extraction buffer (50 mM Tris, 5 mM EDTA, 10 mM monothioglycerol, pH 8.0). The homogenate was left on ice for 10 min and subsequently centrifuged at 15,000 g for 30 min at 4°C. The pellet was used for desmin analysis (see below), and an aliquot of the supernatant was mixed with glycerol to a final concentration of 30% and stored at -80°C until analysed for calpain activity by casein zymography (Pomponio *et al.*, 2008). A reference standard was made from the no ageing (day 2) samples by mixing 1 ml of the supernatant from each animal in the experiment. Glycerol was added to a final concentration of 30% and the reference was stored at -80°C until use on each zymography gel. For desmin analysis the pellets were resuspended in 20 ml of washing buffer (50 mM Tris, 5 mM EDTA, pH 8.0) and centrifuged at 1500 g for 10 min. The myofibrillar pellets were resuspended in 10 ml of washing buffer and protein concentrations were determined by BCA protein assay kit (Pierce Laboratories, Rockford, IL) and adjusted to 3.0 mg/ml of total protein in sample buffer (247 mM Tris, 2% LDS, 10% glycerol, 320 mM dithiothreitol, 0.51 mM EDTA, 0.02% bromophenol blue). Samples were heat denatured for 10 min at 70 °C and loaded onto NuPAGE 10% Bis-Tris polyacrylamide gels (15 wells per gel, Invitrogen, Carlsbad, CA). Onto each lane 15 µg of

protein was loaded. Electrophoresis and Western blotting was performed as described (Ertbjerg *et al.*, 1999) with the following modifications. Gels were run at a constant voltage setting of 200 V for 50 min using MOPS running buffer. Proteins were transferred at 30 V for 1 h onto 0.2- $\mu$ m pore size polyvinylidene difluoride membranes (Invitrogen, Carlsbad, CA). Membranes were probed with mouse monoclonal anti-desmin antibody (clone DE-R-11, DakoCytomation, Glostrup, Denmark) diluted 1:5000 in Tris buffered saline containing 0.05% Tween-20 (TBS-T) and 1% non-fat dry milk. Bound desmin antibody was labelled with sheep anti-mouse IgG horseradish peroxidase-conjugated secondary antibody (NA931V, GE Healthcare, Little Chalfont, UK) diluted 1:5000 in TBS-T containing 1% non-fat dry milk. Desmin was visualized using chemoluminescent substrate (ECL Plus Western Blotting Detection System, GE Healthcare) and intensity of bands were analysed by the Phoretix 1D system (Nonlinear Dynamics, Newcastle upon Tyne, UK).

#### *Protein oxidation*

Protein oxidation was assessed by measuring carbonyl groups formed during incubation with 2,4-dinitrophenylhydrazine (DNPH) in 2M HCl according to the method described by Olivier *et al.* (1987). The method was slightly modified by measuring protein and carbonyl concentrations in the same extract, as the absorption peaks did not overlap (Lindahl *et al.*, 2010). The samples were analysed in duplicates. The carbonyl concentration was calculated by measuring DNPH incorporated on the basis of absorption of  $21.0 \text{ mM}^{-1}\text{cm}^{-1}$  at 370 nm for protein hydrazones. Protein concentration was analysed by measuring the absorption at 280 nm using bovine serum albumin (BSA) as standard. Protein oxidation was expressed as nmol carbonyls/mg protein.

## Statistical methods

Statistical evaluation was performed using Proc Mixed in Statistical Analysis System (version 9.1, SAS Institute Inc., Cary, NC, USA). Degrees of freedom were estimated with the Satterthwaite method (Paper II-IV).

### *Paper I*

The model used for shear force and water loss included the fixed effects of ageing time, treatment (chilled or frozen), their interaction, and animal as a random effect. For the sensory results, the model included treatment as a fixed effect, and animal and panellist as random effects. A Chi-square test was performed on the results from the consumer test.

### *Paper II*

The MIXED procedure was applied to calculate least-squares means (LSM) and standard errors (SE). The statistical model for sample weight included ageing system and location at LD as fixed effects and animal as random effect. The statistical model for shear force and cooking loss included ageing system and location at LD as fixed effects, animal as random effect and sample weight as covariate. To correct for unequal variances in the different treatments, Satterthwaites method was implemented using treatment groups in the repeated statement. The statistical model for purge loss included ageing system as fixed effect, animal as random effect and sample weight as covariate. The model for m-calpain, desmin and carbonyls included ageing system as fixed effect and animal as random effect. The option PDIFF was used to calculate *P* values when testing differences between LSMs. We selected the most interesting comparisons, Control vs. Treatments and difference between ageing systems within the same ageing time. To adjust for multiple comparisons by using the Bonferroni method, obtained *P* values were multiplied by the number of comparisons.

### *Paper III*

The MIXED procedure was applied when calculating least-squares means (LSM) and standard errors (SE) and the option PDIFF was used for calculating significant differences between LSM. The model used for shear force, water loss,  $\alpha$ -tocopherol and protein oxidation included treatment as fixed effect and animal as random effect. To evaluate differences between animals in oxidation pattern, animals were also included as fixed factor in a separate model. The model used for the sensory analysis included treatment as fixed effect, and animal and panellist as random effects. The model for

colour parameters included treatment and display time and their interaction as fixed effects and animal as random effect. For estimating differences in colour parameters between treatments at each display time a model including treatment as fixed effect and animal as random effect was used.

*Paper IV*

Least-squares means (LSM) and standard error (SE) were calculated. A mixed model was used with treatment as fixed factor and animal as random factor. For sensory analysis, panel member was also included as additional random factor. The difference between least-squares means were calculated using the PDIFF option.

## Summary of presented papers

### Paper I

To study how meat quality differ between beef stored either chilled or frozen before cooking, both LD muscles from eight young Holstein bulls were cut into eight samples, weighed, vacuum packed and aged at 4°C for 2, 7 or 14 days. Meat tenderness was analysed instrumentally using Warner-Bratzler shear force and sensorially using both a consumer panel and a semi-trained panel. After ageing, the frozen samples were kept at -20°C prior to heat treatment. Water holding capacity was recorded as purge or thawing loss and cooking loss or as combined loss. Sensory analyses were performed on samples aged 7 days. Peak force values declined with ageing time and freezing. Frozen meat aged 2 days had the same peak force values as chilled meat aged 7 days. Total energy was the same for both treatments at day 2 and 7, whereas at day 14 frozen samples showed significantly higher values than chilled samples. The sensory panel experienced the chilled meat to be more tender, juicier and having a more intense meat taste than the frozen meat, whereas the consumers found no significant difference in degree of liking. Water holding capacity was lower for the frozen samples. The results indicate that conclusions from studies concerning sensory quality of beef depend on whether the meat has been kept chilled or frozen before testing and that this should be taken into consideration when planning a trial.

### Paper II

The aim was to investigate the effect of ageing large beef cuts directly in high-oxygen modified atmosphere packaging (MAP) and how ageing time in vacuum influence meat quality when followed by retail packaging in MAP. Large cuts (10 cm long) of LD muscles from both side of the carcass were aged for up to 25 days postmortem in different ageing systems at 4°C. Ageing solely in high oxygen modified atmosphere (80% O<sub>2</sub> and 20% CO<sub>2</sub>) for 5 or 10 days and ageing in vacuum for 5 or 15 days followed by high oxygen MA for 5 or 10 days were compared with ageing in vacuum for 5, 15 and 25 days. Warner-Bratzler shear force, purge and cooking losses, calpain activity and desmin and carbonyl contents were measured. Shear force decreased to the same level when ageing this large beef cut solely in high-oxygen MAP for 5 or 10 days as when ageing in vacuum. The activity of  $\mu$ -calpain disappeared, the activity of m-calpain decreased and purge loss increased between 5 and 10 days, but cooking loss and the contents of

desmin and carbonyls were unaffected. The ageing time in vacuum before packaging of this large beef cut in high oxygen MA did not affect the ultimate shear force. The m-calpain activity decreased and the content of carbonyls increased compared with solely in vacuum after 15 days of total ageing, but there was no difference in the content of desmin or cooking loss between these ageing systems at the same ageing time.

### Paper III

To determine how sensory quality, shear force, colour and water loss differ between beef steaks in MAP (80% O<sub>2</sub> and 20% CO<sub>2</sub>) and vacuum packaging LD muscles from both sides of ten young bulls were cut in steaks day 3 *postmortem*. The steaks were either frozen directly or after storage at 4°C for 5 or 15 days in MAP and/or vacuum. The parameters studied were Warner-Bratzler shear force, colour stability,  $\alpha$ -tocopherol content, protein oxidation, water holding capacity and sensory attributes. Shear force decreased after ageing for steaks stored only in vacuum. Steaks stored in MAP had higher shear force than steaks stored in only vacuum at all ageing times and did not differ significantly from samples frozen 3 days after slaughter. Tenderness, juiciness and meat flavour were negatively affected by storage in MAP. The solely vacuum packaged meat was the most tender and juicy after both 5 and 15 days storage. The ageing time influenced the colour stability during the following display of steaks in air. Ageing in MAP induced higher levels of metmyoglobin compared with vacuum ageing. Steaks aged for 15 days in vacuum or 5 days in vacuum followed by 10 days in MAP had lower colour stability than steaks aged for shorter times. In conclusion, high-oxygen MAP negatively influences shear force, thawing loss,  $\alpha$ -tocopherol content and colour stability, as well as the sensory attributes tenderness, juiciness and meat flavour.

### Paper IV

To assess how beef quality traits are affected by skin packaging compared with vacuum and high-oxygen MAP (80% O<sub>2</sub> and 20% CO<sub>2</sub>). LD muscles from both sides of six young bulls were cut from the carcasses 1 day postmortem, vacuum packed and aged 7 days. The LDs were then cut into 3.5 cm steaks and either frozen directly or stored in skin pack, vacuum pack or MAP for an additional 7 or 14 days and then frozen (-20°C). Shear force and raw and cooked meat colour were measured instrumentally and the sensory attributes tenderness, juiciness, acidity and meat flavour were assessed. The results showed no differences between skin pack and vacuum pack for shear force and total loss, but skin packed steaks had lower purge

loss. Moreover, no significant differences were found between skin packed and vacuum packed steaks for the sensory attributes tenderness, juiciness and meat flavour. MAP steaks had lower tenderness scores than the other treatments at day 14 and 21 and tenderness did not differ from the initial day 7 samples. MAP steaks had lower scores for juiciness and meat flavour than skin packed and vacuum packed steaks and these attributes decreased with longer storage time in MAP. Raw MAP steaks were bright red, but showed signs of premature browning when cooked.

## General discussion

Centralized packing of meat was introduced to increase shelf life compared to meat wrapped in PVC. Today case-ready packaging of beef in MAP with high-oxygen content is widely used in the retail market (Carpenter *et al.*, 2001) and offers a uniform cost-effective high-quality controlled product at a low distribution cost (Jeyamkondan *et al.*, 2000), packaged in a controlled environment. The relation between consumer perception of quality and the food industry's drive to satisfy consumer needs is a difficult balance since the consumer perceptions are dynamic and sometimes difficult to define (Troy & Kerry, 2010). Consumers base their purchasing choices on perceived quality and the bright red colour of beef in high-oxygen MAP is to many a sign of freshness and good meat quality, making colour the most important quality attribute for retailers. This is the main reason why the amount of centralized packaging of red meats has increased so much. It looks attractive and red in the meat counter of the super markets and the adding of CO<sub>2</sub> to the gas composition of the head space gives the meat a longer shelf life. As an example, minced meat will have an increased shelf life from 1 day when wrapped in PVC to 10 days in high-oxygen MAP (pers. comm., J. Gyllsdorff). The long shelf life is convenient for many consumers since they do not have to cook their minced meat the same day as it was bought. However, while the bright red colour is favourable for consumers at the moment of purchase, the high levels of oxygen promotes oxidation of both proteins and lipids, giving them an inferior product compared with packaging systems that exclude oxygen.

## Beef packaged in high-oxygen MAP

The successes of MAP for packaging of meat depends on many factors including good hygienic conditions during processing and packaging steps in the production plant together with consumer convenience (Singh *et al.*, 2011). Most of the shelf life properties of beef are extended by use of MAP; however, to get the bloomed red OxyMb colour favoured by consumers at the moment of purchase, oxygen needs to be added to the head space (McMillin, 2008; Shay & Egan, 1987). The shelf life of high-oxygen MAP is not as long as that in vacuum packages but it is still about twice that obtained in air (Shay & Egan, 1987). Nonetheless, the high-oxygen content may promote oxidation of lipids, proteins and pigments in the meat, which leads to inferior beef quality.

The deterioration of the sensory quality for beef steaks packaged in high-oxygen MAP was found in both Paper III and IV compared with vacuum and skin pack. In Paper III, vacuum packed steaks were perceived as more tender than high-oxygen MAP steaks after both 5 and 15 days of storage. MAP steaks in Paper IV had the same tenderness scores as the 7-day samples after both 14 and 21 days of storage, indicating no tenderization effect in MAP. Lower tenderness scores for beef when it was exposed to high-oxygen MAP compared to anaerobic packaging systems were also found in other studies (Kim *et al.*, 2010; Clausen *et al.*, 2009; Clausen, 2004; Tørngren, 2003). In both Paper III and IV, MAP steaks also had lower scores for meat flavour and juiciness compared with vacuum- and skin-packed steaks. The decreased juiciness for MAP steaks was also combined with a higher total water loss. In contrast to our results, reduced juiciness scores for steaks in high-oxygen MAP could not be explained by weight loss in the study by Clausen *et al.* (2009). The lower WHC in Paper III might be influenced by an increased level of protein oxidation causing limited degradation of cytoskeletal proteins and hence increased shrinkage of the overall muscle cell (Huff-Lonergan & Lonergan, 2005), even though this was not verified by our protein oxidation analysis.

Although off-flavour was not measured in Paper IV, comments from the sensory panel indicated an increase in rancid, stale flavour with increased storage time in high-oxygen MAP, whereas the other treatments showed no indications of off-flavour. Moreover in other studies storage of beef in high-oxygen MAP generated high off-flavour or WOF or both (Kim *et al.*, 2010; Clausen *et al.*, 2009; Clausen, 2004). Beef packaged in high-oxygen MAP resulted in a large increase in WOF and TBARS as well as a decrease in

juiciness and tenderness compared with packages without oxygen (Clausen *et al.*, 2009). The correlation between sensory analysis and TBARS has been found to be high; consequently TBARS is a good predictor of the perception of rancidity (Campo *et al.*, 2006). After storage in high-oxygen MAP, several volatile compounds, mainly carbonyls such as ketones and aldehydes from the lipid oxidation, were identified to be responsible for the rancid flavour in beef, using gas chromatography–olfactometry (Resconi *et al.*, 2009). The lower tenderness and juiciness scores found in beef steaks packed in high-oxygen MAP may be due to protein oxidation leading to cross-linking/aggregation of myosin, and hence a deterioration in sensory quality (Kim *et al.*, 2010).

In a consumer test, Nordic consumers from Norway, Denmark and Sweden all preferred steaks that were packaged without oxygen, in terms of overall liking, willingness to pay and sensory quality (Aaslyng *et al.*, 2010). This result indicates that the deterioration of sensory quality found by our sensory panel is also noticeable for consumers. In conclusion the high colour stability in the package of high-oxygen MAP cannot compensate for the decrease in tenderness, meat flavour and juiciness.

## **Beef packaged in vacuum and skin pack**

To achieve adequate tenderness for beef, refrigerated ageing for a minimum of 7 days has been implemented by the Swedish meat industry. To achieve this ageing with little spoilage and no surface drying, vacuum packaging is a good solution since it also clearly indicates to the packer when the process has failed and there is a risk of spoilage (Smulders *et al.*, 2006). Vacuum packages are commonly used for packaging whole muscles for the initial ageing period before they are cut and packaged in consumer packages. The considerable advantage of meat packaged in vacuum is that the tenderisation process continues in the package leading to more tender meat. The vacuum packages are easy to handle and store, and the long shelf life may prevent the need for short-time frozen storage (Resurreccion, 2003).

The two main problems with selling meat to consumers in vacuum packages are i) the colour, since the meat has a purple DeoxyMb colour and ii) the amount of purge in the vacuum packages, which does not look appealing to consumers. The lack of the bright red colour of skin packed beef was regarded as a possible disadvantage in marketing (Taylor *et al.*,

1990). However, the purple red DeoxyMb colour of skin pack was stable and remained constant for a 28-day storage period (Taylor *et al.*, 1990). This long-term colour stability was considered an advantage and the meat was still able to bloom when exposed to air. With a self-sufficiency rate for beef and veal of approximately 60%, Sweden has a large import of beef mainly from EU countries but even from South America. The imported beef from South America is much older than the Swedish beef and has low colour stability and is therefore kept in their vacuum packages in the supermarkets. This has to some extent opened up the market for Swedish beef in vacuum packages, making the consumers more used to this packaging type (pers. comm. J. Gyllsdorff).

The decline in shear force values with longer ageing time in paper III and IV for vacuum- or skin packed-steaks was expected and agrees with other studies (Enfält *et al.*, 2004; Monson *et al.*, 2004; Shanks *et al.*, 2002; Crouse & Koohmaraie, 1990). Both vacuum packed (Paper III and IV) and skin packed steaks (Paper IV) had higher tenderness scores compared with that increased with longer storage time which was not the case for high-oxygen MAP steaks. Previous studies have, however, shown that skin packed-steaks were less tender than vacuum-packed steaks (Clausen *et al.*, 2009; Vázquez *et al.*, 2004) or that they were tougher numerically although not significantly different (Barros-Velázquez *et al.*, 2003). Beef texture was not affected when comparing storage in high-oxygen MAP (75% O<sub>2</sub> and 25% CO<sub>2</sub>) and skin pack after an initial ageing period of 13 days post-slaughter, however, there was a large animal depending variation for mean shear force values (Taylor *et al.*, 1990). In our study both shear force and sensorially perceived tenderness differed between skin pack and MAP, but our initial ageing period was 7 days. The fact that we found no differences in tenderness between vacuum and skin pack is interesting since others have. One explanation for this might be different vacuum pressure during packaging. It was observed that skin packed steaks were thinner than the other steaks after storage before freezing (Figure 6). However, this difference in height was not significant when measured after thawing.

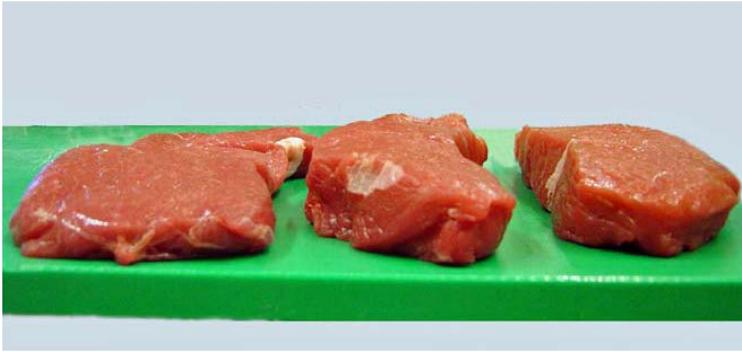


Figure 6. Observed differences between treatments in thickness of steaks from the same animal after storage for 14 days. From left to right: skin pack, vacuum pack and MAP.

One of the advantages of skin pack is that the method is supposed to give less purge loss in the package compared to vacuum packing. In our study, skin pack had the lowest amount of purge loss during storage and vacuum-packed steaks had the most. However, total loss did not differ (including purge, thawing and cooking loss) between skin pack and vacuum pack steaks. In contrast to our results, Vázquez *et al.* (2004) found a higher water loss in skin pack compared with vacuum packed beef. In another study (Clausen *et al.*, 2009) steaks in skin pack had lower water loss during storage compared with vacuum packed steaks after 20 days of storage. Lower purge loss gives less fluid that can be substrate for bacterial growth. The microbiological status has been shown to be better in skin pack compared to vacuum package, which could suggest that skin pack is a more desirable packaging solution. A low purge loss in the package is also positive for the appearance of retail displayed beef. In the study by Taylor *et al.* (1990) the mean drip loss in high-oxygen MAP was generally low up to 6 days of storage after which the drip loss increased, whereas drip loss remained generally low in skin packed samples for the entire trial period of 30 days.

Another advantage of skin packed meat put forward by consumers is the lack of off-odour when the package is opened. The odours that can be detected when opening vacuum packs have been described as sour, acid or cheesy (Dainty *et al.*, 1979). The predominating bacteria in vacuum and skin pack meat packs are lactic acid bacteria (Taylor *et al.*, 1990; Dainty *et al.*, 1979). Lactic acid bacteria and aerobic mesophiles grew slower on skin packed meat compared with vacuum packed meat giving a longer shelf life for skin packed meat (Barros-Velázquez *et al.*, 2003). Spoilage bacteria in skin packed meat grew slowly, if at all, thus resulting in a long odour-free

shelf life (Taylor *et al.*, 1990). In their study, samples in high-oxygen MAP developed off-odours between 8 and 12 days of storage, whereas off-odours were almost absent for skin packed beef after 28 days of storage. One explanation for these results might be the instantaneous thermal treatment when the upper plastic film is applied onto the meat, thus reducing the bacterial load on the meat surface (Barros-Velázquez *et al.*, 2003). The smaller amount of purge loss in the skin packs might also contribute to the less amount of off-odour. When adding the gas mixture 25% CO<sub>2</sub>/65% N<sub>2</sub>/10% O<sub>2</sub>, pork chops had more surface greening, stronger off-odour and psychotropic counts after storage compared with anaerobic packaging methods (Sørheim *et al.*, 1996).

The results of study IV show no clear differences between skin packed and vacuum packed steaks for shear force and total loss; however, skin packed steaks had lower purge loss which might be more appealing to the consumers in retail display. The problem with unattractive purge in the packages could be solved by packaging the meat in skin pack; however, this type of packaging solution is more expensive (pers. comm., A. Lundell). Furthermore, skin packed and vacuum packed steaks did not differ for the sensory attributes tenderness, juiciness and meat flavour. In contrast, high-oxygen MAP negatively influenced shear force and water-holding capacity as well as the sensory attributes tenderness, meat flavour and juiciness compared with beef steaks packaged in skin pack or vacuum. In a consumer study, Swedish consumers preferred skin packed steaks and were willing to pay more than usual compared with high-oxygen MAP (Aaslyng *et al.*, 2010), meaning that even though this type of package is more expensive there is a market for it.

## Colour

High colour stability is essential for the meat industry since the colour gives the consumer an indication of the meat quality at purchase (Troy & Kerry, 2010). There are many factors affecting meat colour such as genetics, diet and the accumulation of antioxidants such as  $\alpha$ -tocopherol as well as pH and chilling rate (Mancini & Hunt, 2005). To achieve a stable bloomed OxyMb colour retail packaging of meat in high-oxygen MAP needs an oxygen content of at least 55% (Jakobsen & Bertelsen, 2000). Shelf life is prolonged through the inhibition of bacterial growth if 20-30% CO<sub>2</sub> is added (McMillin, 2008). The bright red OxyMb colour depends on the rate of O<sub>2</sub>

diffusion and O<sub>2</sub> consumption and on the partial pressure of O<sub>2</sub> at the meat surface. The prolonged colour stability in high-oxygen MAP compared with air depends on the formation of a thicker layer of OxyMb at the meat surface that masks the underlying MetMb layer (Jeremiah, 2001). The high content of oxygen in the MAP headspace compared to the oxygen content of air, has been observed to preserve OxyMb colour longer and have a delaying effect on MetMb formation (Jeremiah, 2001).

In both paper III and IV beef steaks were stored in air for 5 days after their storage times in the different packaging solutions. In paper III ageing in MAP induced higher levels of MetMb compared with vacuum ageing during the following display of steaks in air. Steaks aged for 15 days in vacuum or 5 days in vacuum followed by 10 days in MAP had lower colour stability than steaks aged for shorter times. In Paper IV the raw meat in MAP had higher a\* values compared with vacuum and skin packed steaks and a\* and b\* values did not differ between vacuum and skin pack steaks. However, when the meat was cooked the L\*, a\* and b\* values for MAP steaks were lower compared with vacuum and skin packed steaks. MAP steaks showed a well-done appearance at 72-73°C whereas vacuum and skin packed steaks had an attractive pink core colour.

In paper III, the initially higher level of MetMb during storage for MAP steaks compared with vacuum aged steaks was expected since the study was designed so that oxygen could penetrate throughout the steaks. This was also reflected in lower redness compared with vacuum aged steaks during the first days of storage in air. The level of MetMb increased only slightly during 5 days of storage in air after ageing in MAP for 5 days. Longer storage time, 15 days, induced the highest formation of MetMb and thus the lowest colour stability during storage irrespective of packaging method. Meat colour stability is the net result of autoxidation and reduction of MetMb. The MetMb-reducing activity is known to decrease postmortem (Mancini & Hunt, 2005; Tang *et al.*, 2005), which could explain the high MetMb content after 15 days of ageing. The lower content of  $\alpha$ -tocopherol in high-oxygen MAP steaks could also explain the higher level of MetMb compared with vacuum in Paper III, since  $\alpha$ -tocopherol is involved in the regeneration of MetMb-reductase (Lynch *et al.*, 1998). In the study by Clausen *et al.* (2009) the  $\alpha$ -tocopherol content rapidly decreased during storage due to the exposure to high-oxygen MAP. In Paper III, the  $\alpha$ -tocopherol content did not differ significantly between day 0 steaks and the vacuum stored steaks, but high-oxygen MAP steaks had significantly lower

$\alpha$ -tocopherol content. Changes in OxyMb, L\* and a\* values appeared to be driven by the oxidation of lipids and correlated strongly with TBARS (Zakrys *et al.*, 2008). Raw meat with less than approximately 2 mg  $\alpha$ -tocopherol/g meat showed a very high degree of lipid oxidation with TBARS and WOF values after 20 days of storage in 50% O<sub>2</sub>/CO<sub>2</sub>. The likely mechanism for the colour stabilising effect of  $\alpha$ -tocopherol is through its action as a lipid antioxidant (O'Grady *et al.*, 2000; Faustman *et al.*, 1998).

### Premature browning

Many consumers use the internal cooked appearance as a way of evaluating doneness (King & Whyte, 2006; Hunt *et al.*, 1999), particularly for minced meat patties. If the raw patties contain mostly MetMb or OxyMb before cooking, a brown well-done appearance, premature browning (PMB), develops at the temperatures low enough for pathogenic bacteria to survive (Hunt *et al.*, 1999). The concept of PMB has therefore become more and more important as the amount of meat packaged in high-oxygen MAP with a high level of OxyMb increases. Raw MAP steaks in Paper IV were bright red and had higher a\* values at both days 14 and 21 compared with skin pack and vacuum packed steaks. After cooking the steaks in MAP in the same study had a greyish surface colour and a grey internal colour at 72–73°C, whereas steaks in vacuum or skin pack had a brown exterior and a slightly pinkish interior. The internal well-done appearance and external grey colour in MAP was probably due to premature browning phenomenon. In Paper II, the 1.5- to 2-cm-thick layer that oxygen had been able to penetrate during storage in high-oxygen MAP was bright OxyMb red before cooking and developed a well-done appearance when cooked (Figure 7).



Figure 7. The 1.5- to 2-cm layer of OxyMb prior to and after cooking.

Consumers found the high-oxygen MAP steaks to have a too well-done appearance compared with steaks packaged without oxygen in a study by (Aaslyng *et al.*, 2010). In another study (Tørngren, 2003), sensory evaluation of internal colour after cooking showed that anaerobic ageing resulted in a satisfactory pink colour, whereas meat stored in high-oxygen MAP had a well-done appearance. In a different study (Hunt *et al.*, 1999), ground beef patties with predominantly OxyMb had a well-done appearance at the endpoint temperature 55°C and could be mistakenly perceived as cooked enough to consume. The study also found that patties with DeoxyMb, found in aerobic packaging methods, did not show a well-done appearance until 75°C.

## Protein oxidation

Packaging beef steaks in high-oxygen MAP was found to increase shear force compared with beef steaks packaged only in vacuum (Paper III & IV). In Paper II, oxygen did not penetrate throughout the meat in the large 10-cm piece of LD, but only to 1.5-2 cm depth and shear force was not affected. Our results from Paper III and IV agree with those from other studies (Zakrys *et al.*, 2008; Sørheim *et al.*, 2004). When an increasing amount of oxygen was added in MAP (from 10% to 80%) shear force showed a tendency to increase during 15 days of storage (Zakrys *et al.*, 2008). In Paper III, steaks stored in a combination of vacuum and MAP had higher peak force and total energy values compared with steaks stored in vacuum after 15 days and did not differ significantly from day 0 steaks, thus, erasing the ageing effect on shear force from the time in the 5 days in a vacuum bag. The two main explanations of why meat becomes tougher when stored in high-oxygen MAP have been that i) the enzymes involved in the tenderisation process might be oxidized by the high-oxygen content leading to a slower or interrupted tenderisation process (Rowe *et al.*, 2004) and that ii) intermolecular cross-links involving disulfide bonds are formed in myosin leading to a tougher meat (Kim *et al.*, 2010; Lund *et al.*, 2008; Lund *et al.*, 2007).

A deactivation of  $\mu$ -calpain was found when the meat was oxidised through irradiation (Rowe *et al.*, 2004). In Paper II, the  $\mu$ -calpain activity disappeared with ageing and packaging method had no effect. Lund *et al.* (2011) suggested that high-oxygen MAP produces less strong oxidative conditions as compared to irradiation and therefore there is no observed

effect on  $\mu$ -calpain activity in high-oxygen MAP. However, high-oxygen MAP induced protein oxidation with increased storage time in Paper II, shown as an increased formation of carbonyl compounds, and a reduced amount of extractable m-calpain activity. Nonetheless it did not affect desmin degradation and shear force compared with ageing in vacuum. In Paper II the samples for analysis of calpain activity were taken just inside the visible layer of oxymyoglobin, whereas samples for shear force were taken further inside the piece of LD, which may explain the observed differences in effect on shear force and m-calpain activity. The rate of protein oxidation *postmortem* is known to differ between different muscles (Martinaud *et al.*, 1997) and susceptibility for oxidation may sometimes differ genetically (Huff-Lonergan & Lonergan, 2008). This is in line with the results from Paper III where protein oxidation measured as carbonyls differed significantly between animals, but not between treatments. In Paper II the content of carbonyls was higher for steaks stored in vacuum for 5 days followed by 10 days in MAP compared with controls and steaks stored solely 15 days in vacuum. The used DNPH method for protein oxidation measured as carbonyl concentration is relatively unspecific and there is a need for more specific and suitable methods for analysing protein oxidation (Lund *et al.*, 2011).

The phenomenon of toughening of beef steaks packaged in high-oxygen MAP in both Paper III and IV has even been shown to affect single muscle fibres from beef LD. They had significantly higher breaking strength when packaged in high-oxygen compared with 100% N<sub>2</sub> (Lund *et al.*, 2008). In another study pork stored in high-oxygen atmosphere, myosin heavy-chain (MHC) was found to form intermolecular cross-links (Lund *et al.*, 2007). When muscle is stored postmortem myofibrillar protein oxidation increases (Rowe *et al.*, 2004; Martinaud *et al.*, 1997). This oxidation changes some amino acid residues, including histidine, to carbonyls and can lead to the formation of intra and/or inter protein disulfide cross-links (Martinaud *et al.*, 1997). Both  $\mu$ -calpain and m-calpain enzymes are particularly susceptible to inactivation by oxidation as they contain both histidine and SH-containing cysteine residues at their active sites (Lametsch *et al.*, 2008). The cysteine residues of the myosin-light chains (MLC) tail region are located very close to the cysteine residues in the other MLC (Lund *et al.*, 2011) and an oxidation of MLC will promote aggregation and disulfide bonds (Huff-Lonergan *et al.*, 2010). A formation of disulfide bonds in the tail region of myosin are prone to occur during oxidative conditions, a reaction that could explain the observed reduction in tenderness (Lund *et al.*, 2011).

## Sensory analysis and instrumental measurements

Human perception of meat palatability is derived from a complex interaction of sensory and physical processes during chewing. The mechanisms of chewing are complex and difficult to define and are also affected by the secretion of saliva (Dransfield, 1996). The preferred approach has been to standardise the mechanics of an instrumental test and use correlation with sensory data to test its effectiveness (Dransfield, 1996). The relations between mechanical and sensory assessments tend to be non-linear, probably because non-linearity in the sensory evaluation and because muscle fibre orientation is easier to control in instrumental than in sensory evaluation (Tornberg, 1996). Meat tenderness can be evaluated by mechanical devices such as shear force as well as sensory analysis (Tornberg, 1996). Shear force is increasingly favoured over sensory analysis because it is more economical and yields more reproducible results (Larmond & Petrasovits, 1972). Warner-Bratzler (WB) shear is a commonly used instrumental measure of physical properties (Dransfield, 1996; Brady & Hunecke, 1985), which also predicts tenderness (Novakofski & Brewer, 2006). Designing a mechanical test to objectively assess meat tenderness has been difficult since it depends strongly on the test conditions (Dransfield, 1996).

In Paper III, the sensory panel found the vacuum packaged steaks to be the most tender at both day 5 and day 15. However, the high-oxygen MAP steaks had significantly higher tenderness scores at both day 5 and 15 compared with day 0 steaks, whereas shear force analysis, both peak force and total energy, showed no difference between stored high-oxygen MAP steaks and day 0 steaks. In paper IV, the sensory analysis and the measurements of total energy both discriminated better between treatments than measurements of peak force although the sensory analysis was most discriminating in respect to day 0 steaks. In Paper I, correlations were higher between peak force and tenderness for the frozen samples than for the chilled samples, probably due to the influence of the higher juiciness scores for the chilled meat. In a study by Wheeler *et al.* (2004) a large portion of the untrained laboratory consumer panel could identify differences in beef tenderness on a scale from 1 to 8, in a controlled laboratory environment. The tenderness ratings from their laboratory consumer panel were strongly correlated to the peak force measurements. In a study by Desdefanis *et al.*

(2008) the correlation coefficient of WB measurements with tenderness sensory ratings was found to be  $-0.72$ . Of the consumers, 55.6% could omit tough from intermediate and tender meat and 62.3% distinguished tender from intermediate and tough meat. For tenderness we have observed that it is fairly easy for untrained panel members and consumers to observe differences. However, other sensory traits such as meat flavour and juiciness need more training to get good results.

Although meat tenderness is usually assessed mechanically, meat characteristics are of no value if they are not confirmed by sensory evaluation as only this will reflect the exact eating experience during meat consumption (Ruiz de Huidobro *et al.*, 2005). However, it is essential that the results of the panel is as accurate and exact as possible (Ruiz de Huidobro *et al.*, 2005). Sensory analysis can be expensive and time consuming, and an instrumental measurement can be a good complement. The sometimes poor correlation between shear force and sensory analysis may be because instructions to assessors have not been clearly defined (pers. comm., G. Nute). Texture profile analysis through measurement of compression force is rarely used for meat but was better at predicting sensory variants compared with shear force (Ruiz de Huidobro *et al.*, 2005; Ruiz de Huidobro *et al.*, 2005). Texture profile analysis was found to better predict hardness, juiciness and number of chewings compared with shear force.

Even if WB peak force is one the most common instrumental measurements, it is perhaps not the best to predict sensorially perceived tenderness. The findings for instrumentally measured tenderness and sensory quality often agree when measured in the same study but not always. The lack of agreement between sensory analysis, peak force and total energy in Paper I is one example. The divergent results could be due to the fact that instrumentally evaluated shear force measure partially different characteristics in comparison with sensorially measured tenderness. Moreover possibly the slightly different procedures in freezing and thawing time contributes to the difference. The different ways of cooking the samples in Paper I for shear force and sensory analysis may also have affected the result; however, the final inner temperature was the same. Our results from Paper I indicate that studies concerning sensory quality of beef meat depend on whether the meat has been kept chilled or frozen before testing and show that such testing should preferably be conducted on chilled meat. Although this is not always possible for practical reasons, it should be taken into consideration.

## Main conclusions

The results presented in this thesis give an insight into what happens with the quality of beef when it is packaged and stored in high-oxygen MAP or vacuum and skin pack. It also shows how freezing effects beef quality. The main conclusions are:

- High-oxygen MAP systems for beef steaks negatively influenced shear force and water-holding capacity as well as the sensory attributes tenderness, meat flavour and juiciness, compared with beef steaks packaged in skin pack or vacuum. High-oxygen MAP also leads to decreased  $\alpha$ -tocopherol values and increased protein oxidation.
- The colour of beef in high-oxygen MAP is bright OxyMb red, but it has a lower colour stability compared to vacuum when stored for 5 days in air, and develops a well-done colour when cooked.
- When a 10 cm piece of beef was packaged in high-oxygen MAP shear force was not effected since the measurements were done inside the visible layer of OxyMb. This would indicate that the negative effect on beef in high-oxygen MAP to some extent could be avoided if the piece is so large that the oxygen is not able to penetrate the whole piece. However, when the meat was cooked, the OxyMb layer developed a well done appearance that may be discouraging to some consumers.
- WB shear force values declined with longer storage time for beef steaks packaged in vacuum or skin pack and the beef steaks were perceived as being more tender by the sensory panel. This was not the case with steaks in high-oxygen MAP.

- No clear differences was found between skin packed and vacuum packed steaks for shear force and total water loss, however, skin packed steaks had lower purge loss which might be more appealing to the consumers in retail display.
- The inferior quality of beef in high-oxygen MAP suggests that oxygen should be excluded from the head space gas composition or that beef steaks should be packaged in vacuum or skin pack.
- Freezing negatively effects sensory quality of meat, since the higher loss of water affects the overall mouth feel and the chilled meat is perceived as being more tender. Most research is done on frozen meat due to practical reasons. As consumers usually eat meat fresh, the differences in quality that comes with freezing should be taken into consideration when planning research.

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