Genetic and Ageing Effects on Beef Quality

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Cover: cross sections of beef *longissimus* muscle showing different levels of intramuscular fat content (marbling).
(photo: Xin Li)

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Abstract

The overall aim of this thesis was to investigate genetic and ageing effects on beef quality.

To study the genetic effects, association analyses were carried out between single nucleotide polymorphisms (SNPs) at *DGAT1*, *LEP*, *SCD1*, *CAPN1* and *CAST* genes with colour, marbling, water holding capacity (WHC) and tenderness in meat from young bulls of the beef cattle population in Sweden. In total 243 young bulls from five beef breeds were included in the analysis. The results confirmed previously reported associations of the K232A polymorphism in the *DGAT1* gene with marbling and polymorphisms in *CAPN1* and *CAST* genes with meat tenderness. The *LEP*, *SCD1* and *CAPN1* genes showed association with meat colour traits. Our results confirm that part of the variation in these beef quality traits is under genetic control. Although our analyses revealed only minor breed differences, the lack of consistent gene effects between studies with different breeds indicate that association between markers and traits may not be consistent over breeds, possibly due to different genomic background. This may have implications on the applicability across populations of SNP chips developed in only one or a few breeds.

To assess the ageing effects, three ageing methods, dry ageing bag, vacuum ageing and traditional dry ageing were compared. The beef muscles *longissimus thoracis et lumborum* and *gluteus medius* were used for the ageing process. The results showed that the dry ageing bag, as an alternative to traditional unpackaged dry ageing, made it possible to decrease meat ageing loss and microbial growth compared with traditional dry ageing. Meat aged in dry ageing bags has similar sensory characteristics as traditional dry ageing for most of the sensory attributes. Compared with vacuum ageing, the total product yield was lower after ageing meat in dry ageing bags. However, the dry ageing bags produced meat with enhanced tenderness and juiciness, characteristics that are valued by consumers. Thus, by using dry ageing bags it is possible to produce dry-aged meat under more controlled conditions without negative effects on sensory or other quality attributes.

Keywords: beef quality, single nucleotide polymorphism, dry ageing, vacuum

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Dedication

To my supervisor Kerstin Lundström

Contents

List of Publications

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

I **Li, X.**, Ekerljung, M., Lundström, K., Lundén, A. Association of polymorphisms at *DGAT1*, leptin, *SCD1*, *CAPN1* and *CAST* genes with colour, marbling and water holding capacity in meat from beef cattle populations in Sweden (submitted).

II Ekerljung, M., **Li, X.**, Lundén, A., Lundström, K., Marklund, S., Näsholm, A. (2012). Associations between candidate SNPs in the calpain 1, calpastatin and leptin genes and meat tenderness among Swedish beef populations. *Acta Agriculturae Scandinavica Section A-Animal Science* (accepted).

III **Li, X.**, Babol, J., Bredie, W. L. P., Nielsen, B., Tománková, J., Lundström, K. A comparative study of beef quality after ageing *longissimus* muscle using a dry ageing bag, traditional dry ageing or vacuum packing (submitted).

IV **Li, X.**, Babol, J., Wallby, A., Lundström, K. Meat quality, microbiological status and consumer preference of beef *gluteus medius* aged in a dry ageing bag or vacuum (submitted).

The contribution of Xin Li 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 part of the laboratory work. Was responsible for the statistical evaluation of the results and for writing the manuscript.

II Participated in the planning of the experimental work together with supervisors. Participated in the collection of samples and performed part of the laboratory work. Participated in the statistical 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 colour measurements and water content analysis. Participated in the preparation of samples for microbiology and sensory evaluations. Participated in the statistical 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 laboratory work. Participated in the statistical evaluation of the results and was responsible for writing the manuscript.

Abbreviations

CAPN1	μ-calpain gene
CAST	Calpastatin gene
DeoxyMb	Deoxymyoglobin
DGAT1	Diacylglycerol O-acyltransferase 1 gene
EB	*Enterobacteriaceae*
GM	*Gluteus medius*
LAB	Lactic acid bacteria
LEP	Leptin gene
LSM	Least-squares means
LT	*Longissimus thoracis*
LTL	*Longissimus thoracis et lumborum*
MetMb	Metmyoglobin
MUFAs	Monounsaturated fatty acids
NADH	Reduced nicotinamide adenine dinucleotide
OxyMb	Oxymyoglobin
PCA	Principal Components Analysis
SCD1	Stearoyl-CoA desaturase 1 gene
SD	Standard deviation
SNP	Single nucleotide polymorphism
TBC	Total bacteria counts
WHC	Water holding capacity

1 Introduction

1.1 Meat quality

1.1.1 What meat quality means

Meat quality means different things to different people, which indicates people have different emphasis on meat quality, rather than conflicting opinions on a definition for meat quality. Thus, meat quality is a general concept consisting of several properties and perceptions of meat. A full list of quality characteristics includes the following aspects: (1) yield and composition, e.g. muscle size and shape, quantity of saleable product; (2) appearance and technological characteristics, e.g. colour, marbling, water holding capacity (WHC), chemical composition; (3) palatability, e.g. tenderness, juiciness, flavour; (4) wholesomeness, e.g. nutritional quality, microbiological and chemical safety; and (5) ethical quality, e.g. animal welfare, sustainable and environmental considerations (Warriss, 2000). Consumers usually determine meat quality by its eating quality of which tenderness, juiciness and flavour are the most important elements (Maltin *et al.*, 2003; McIlveen & Buchanan, 2001).

1.1.2 Why meat quality is important

The meat production can be divided into three different periods of development: produce enough to satisfy requirements; make production systems more efficient; and improve the quality of meat products (Warriss, 2000). Nowadays, with the development of the economies worldwide, some countries are in the third period when meat quality becomes important. In the meat industry, farmers' payment, price of meat products and purchase and repurchase decision of consumers are all influenced by meat quality. Therefore, it is necessary to investigate the mechanisms of variations on meat quality and find suitable ways to handle and improve meat quality.

1.1.3 Beef quality characteristics focused on in this thesis

Colour

Consumers usually use meat discoloration as an indicator of freshness and wholesomeness. Meat colour can influence the consumer's satisfaction and purchase decision (Mancini & Hunt, 2005). Consumers visually prefer beef with bright, cherry-red colour to beef with dark red colour (Killinger *et al.*, 2004b). It was estimated that the financial loss caused by meat discoloration was annually one billion dollars in US (Smith *et al.*, 2000).

Meat colour depends on the content and chemical form of the meat pigment and on the physical characteristics of meat, e.g. light scattering and absorbing properties (Kropf, 1993). The principal meat pigment responsible for meat colour is myoglobin (Mb), although other pigments such as hemoglobin and cytochrome C can also affect meat colour (Mancini & Hunt, 2005). Thus, Mb gets the main focus in meat colour research. Myoglobin is a hemeprotein in skeletal muscle that consists of a protein moiety (globin) and a heme prosthetic group. Heme is a complex of protoporphyrin IX and ferrous iron (Fe^{2+}). The iron, located at the centre of the heme molecule, can form six bonds. Four of these bonds are formed with the four nitrogen atoms of the porphyrin ring and the additional two are located on each side of the planar porphyrin ring. The fifth bond coordinates with the proximal histidine residue of the globin and the sixth position is available to reversibly bound ligands. The globin portion of Mb creates a special crevice microenvironment for the heme that enable it to reversibly bind one molecule of oxygen or other small ligands (Harvey & Ferrier, 2011). Myoglobin has three chemical forms, each with its specific colour. Deoxymyoglobin (DeoxyMb) is purple-red with the iron in a ferrous state and no ligand present at the sixth site. This form is associated with the colour of meat stored in vacuum and also with the colour of the muscle surface immediately after cutting. When freshly cut meat is exposed to air, oxygenation, or blooming, occurs during which oxygen binds to the sixth site in DeoxyMb whereby oxymyoglobin (OxyMb) is formed, with its iron still in a ferrous state. The OxyMb gives the meat a bright red colour, which is preferred by consumers. When the heme iron is oxidized to ferric (Fe^{3+}) state and water occupies the sixth site, brown metmyoglobin (MetMb) is formed (Mancini & Hunt, 2005). The formation of MetMb due to myoglobin oxidation on the meat surface is the main cause of meat discoloration. The myoglobin oxidation and lipid oxidation can exacerbate each other through the chemical compounds formed in the oxidation process (Faustman *et al.*, 2010).

Marbling

Marbling refers to the visible appearance of white flecks or streaks of intramuscular fat (IMF) between muscle fibre bundles. The fat present in marbling is an adipose tissue consisting of adipocytes which is embedded in a perimysial connective tissue matrix and is in close proximity to a blood capillary (Hocquette *et al.*, 2010). In the beef industry, the amount and distribution of marbling is often evaluated in the *longissimus* muscle at the cutting surface of the cross section after the carcass has been quartered. The degree of marbling is regarded as an important factor in the beef carcass quality grading system in many countries, e.g. USA, Canada, Australia, Japan and China. However, marbling is not included in the EU beef carcass classification scheme (EUROP).

It is generally accepted that a high degree of marbling positively influences sensory attributes, including juiciness, flavour and tenderness whereas a very low degree of marbling leads to dry and less-tasty meat (Hocquette *et al.*, 2010). As a visible characteristic, marbling is a factor influencing a consumer's purchase decision. Many studies have shown that consumers are willing to purchase and pay more for beef with higher marbling (Platter *et al.*, 2005; Savell *et al.*, 1987), whereas others reported that consumers' visual preference was low-marbled beef (Killinger *et al.*, 2004b). Consumers are willing to pay more for the preferred degree of marbling, irrespective of whether the preference was high-marbled or low-marbled beef steak (Killinger *et al.*, 2004a; Killinger *et al.*, 2004b). Thus, target markets exist for both high and low marbled beef. However, notably the majority of consumers are those preferring high-marbled beef, especially when considering eating quality.

Tenderness

Tenderness is one of the most important elements for evaluating meat eating quality by consumers. Variability of tenderness is the main source of complaints from consumers and the primary cause of failure to repurchase (Maltin *et al.*, 2003). Thus the inconsistency in meat tenderness is a major limitation to consumer satisfaction and addressing the problem is regarded as high priority in the meat industry (Koohmaraie *et al.*, 2002; Tarrant, 1998). Carcasses from bulls are known to have less tender meat and show a larger variation in tenderness than steers (Peachey *et al.*, 2002; Morgan *et al.*, 1993).

Tenderness is a complex trait involving many intrinsic and extrinsic factors. Post mortem events are the main determinants of tenderness (Maltin *et al.*, 2003). After slaughter of an animal, the blood circulatory system is stopped, resulting in failure in the supply of oxygen. The metabolic process can continue for a while under anaerobic condition thereby causing a decrease in

the level of ATP. The actomyosin cross-bridges that are formed as a result of the low ATP level signals the occurrence of rigor mortis which in turn causes an increase in meat toughness (Scheffler *et al.*, 2011). After rigor mortis, there is the tenderization state which makes the meat more tender. The tenderization rate differs among species and is influenced by several factors; therefore different ageing times and processes are applied in meat processing.

Meat tenderization results from post mortem proteolysis. The calpain system is extensively studied and widely accepted as the main contributor to meat tenderization (Kemp *et al.*, 2010). The micro molar calcium activated neutral protease μ-calpain (CAPN1) and its inhibitor calpastatin (CAST) have been shown to be involved in the meat tenderization process (Koohmaraie, 1996). The CAPN1 is the component of the calpain system that plays the most significant role in meat tenderization (Kemp *et al.*, 2010). The CAPN1 is responsible for digestion of desmin and costamere structures during meat tenderization (Maltin *et al.*, 2003).

Water holding capacity

Water is the major component of muscle tissue; on average 75% of the lean meat consists of water. Water holding capacity is the ability of meat to retain its intrinsic water during subsequent manipulations or to take up and hold water added during processing (Offer & Knight, 1988). The WHC of meat is of great economic and technologic importance, not only for the appearance in the store and the cooking properties but also for the meat processing industry. Unacceptable WHC leads to millions of dollars loss in meat industry annually (Huff-Lonergan & Lonergan, 2005).

Losses of water in meat can occur by evaporation and drip and during thawing and cooking (Offer & Knight, 1988). The WHC in this thesis is measured as thawing loss and cooking loss. The mechanism of WHC is centred in the proteins and structures that bind and entrap water, specifically the myofibrillar proteins. The pH decline *post mortem*, ionic strength and oxidation are other factors that have effects on WHC (Huff-Lonergan & Lonergan, 2005).

Microbiology

Undesired microbial growth in meat during storage is the main cause of spoilage of raw meat (Doulgeraki *et al.*, 2012). Microbes that lead to meat spoilage can be bacteria, yeast or mould (Warriss, 2000). There are many intrinsic and extrinsic factors influencing microbial growth in meat. Storage temperature, relative humidity and oxygen are the most influential factors on growth of microorganisms in meat (Hedrick *et al.*, 1994). Because meat

spoilage is not always evident, the consumers usually use strong off-odours, discoloration, the development of slime or best-before date on the package as main qualitative criteria for meat rejection (Nychas *et al.*, 2008).

Growth of a variety of spoilage microbes of importance during meat storage will be discussed in this thesis. Total bacteria counts (TBC) give an indication of the total populations of microorganisms but give little information on the specific organisms present (Hedrick *et al.*, 1994). *Enterobacteriaceae* (EB) is cold-tolerant and able to grow on chilled meat under aerobic storage. The EB counts are considered as indicator of food safety (Nychas *et al.*, 2008). Lactic acid bacteria (LAB) are a group of anaerobic microbes that tolerate high carbon dioxide level, and which can become dominant during storage with reduced oxygen, for example in vacuum package (Doulgeraki *et al.*, 2012). Yeasts, as spores of mould, are unicellular with creamy white colour. Moulds are multicellular organisms and display a variety of colours. Yeasts and moulds can cause spoilage of meat by slimy or fuzzy, cotton-like appearance and by producing off-flavours (Hedrick *et al.*, 1994; Fleet, 1992).

Sensory properties

Sensory properties of meat which are agreeable to the senses make contributions to meat palatability (Hedrick *et al.*, 1994). Important sensory characteristics are texture, juiciness, flavour and odour of the cooked meat (Warriss, 2000). Flavour includes two components: taste and aroma or smell (Warriss, 2000). There are hundreds of compounds in meat that have been identified as contributors to meat flavour. However, many of them are altered through storage and cooking, contributing to the complex nature of meat flavour (Calkins & Hodgen, 2007).

Sensory analysis can be divided into two basic categories: analytical tests and affective tests (Lawless & Heymann, 1999). Analytical tests are usually carried out by a trained sensory panel to objectively obtain the sensory differences of products. Examples of analytical tests are discrimination test and descriptive test. An affective test is intended to determine the consumer's subjective liking of the products. Consumer preference/acceptance tests are typical affective tests. Selection of the specific and appropriate test depends on the specific objective.

The sensory panel evaluation mentioned in this thesis did sensory profiling to describe the subtle differences on sensory properties between beef samples. In addition, consumer tests were carried out to detect preference of consumers on different beef samples.

1.2 Factors influencing beef quality

Many factors have been considered when trying to improve meat quality, such as breed of animal, slaughter age, ageing process and packaging method. It is also possible to influence beef quality utilising genetic information in selection of breeding animals. The main focuses in this thesis are on genetics and ageing process.

1.2.1 Genetics

The rapid development within the field of genomics has stimulated the interest in using molecular tools for meat quality improvement. Heritability is a measure of the proportion of the total variability of a trait in a population caused by genetic factors, relative to environmental factors. In general, genetic variation accounts for 5-30% of the total phenotypic variance in beef quality traits (Warner *et al.*, 2010). Heritability estimates of meat quality traits addressed in this thesis are summarized in Table 1.

Table 1. *Heritability estimates of beef quality traits*

Trait	Heritability	Reference
Colour parameters	0.13-0.41	King *et al.* (2010)
Myoglobin concentration	0.85	King *et al.* (2010)
Marbling	up to 0.65	Marschall (1999)
Shear force	0.17-0.47	Wheeler *et al.* (2005); O'Connor *et al.* (1997)

Genetic markers for meat quality traits can be identified using two approaches, candidate gene approach and quantitative trait loci mapping (Sosnicki & Newman, 2010). In the candidate gene approach, sequencing of candidate loci in individuals representing a large phenotypic variation are used to identify quantitative trait nucleotides, i.e. single nucleotide polymorphisms (SNPs) being the causative mutations for the genetic variation in traits. Several association analysis between various SNPs and beef quality traits have been reported (Mullen *et al.*, 2006). An alternative strategy is to use "genomic selection" (Meuwissen *et al.*, 2001) in which breeding candidates are genotyped for a vast number of markers covering most parts of the genome, and thereby are expected to explain the majority of the genetic variance in a trait. Each SNP is allocated a value that varies between traits, and the sum of all SNP values forms the breeding value. In the long term, models used for estimation of breeding values have been predicted to be based entirely on DNA markers (Goddard, 2009).

Single nucleotide polymorphism

Single nucleotide polymorphism is a type of variation at the DNA level. SNPs are variants that naturally occur and affect a single nucleotide. SNPs can be classified according to the nature of the nucleotide that is affected (Table 2). The SNPs tested in this thesis are located in several candidate genes that are related with beef quality traits.

Table 2. *Classifications of single nucleotide polymorphisms (Gibson & Muse, 2009)*

Classifications	Descriptions
Non-coding SNPs	Located in non-transcribed or untranslated regions or an intron
Coding SNPs	
Replacement polymorphisms	Change the coded amino acid
Synonymous polymorphisms	Change the codon but not the amino acid
Regulatory polymorphisms	Affect gene function

Candidate genes

The diacylglycerol O-acyltransferase 1 (*DGAT1*) gene encodes the microsomal enzyme DGAT1 which catalyses the final step of the triglyceride synthesis. The *DGAT1* gene maps to bovine chromosome 14 and K232A is a non-synonymous dinucleotide substitution in exon 8 where AA codes for the amino acid lysine (K) and GC codes for alanine (A) (Grisart *et al.*, 2002; Coppieters *et al.*, 1998). The K232A polymorphism in the *DGAT1* gene has been shown to be associated with milk fat content (Grisart *et al.*, 2002; Winter *et al.*, 2002) and IMF content in the semitendinosus muscle (Thaller *et al.*, 2003).

The leptin (*LEP*) gene, also called the "obese gene", located on bovine chromosome 4, encodes leptin, a peptide hormone, which is synthesized and secreted by adipose tissue (Stone *et al.*, 1996; Zhang *et al.*, 1994). The concentration of leptin could be seen as an indicator of marbling, back fat depth, and yield and quality grade in feedlot cattle (Geary *et al.*, 2003; McFadin *et al.*, 2003). Markers at promoter regions of the bovine *LEP* gene have been shown to be associated with marbling and carcass traits (Nkrumah *et al.*, 2005).

Stearoyl-CoA desaturase 1 (SCD1) is an enzyme responsible for catalysing the desaturation of saturated fatty acids to monounsaturated fatty acids (MUFAs). Polymorphisms at the *SCD1* gene have been shown to be associated with beef fatty acid composition (Taniguchi *et al.*, 2004), IMF deposition (Wu *et al.*, 2012), and meat colour traits (Reardon *et al.*, 2010).

The *CAPN1* gene locates on bovine chromosome 29 and the *CAST* gene locates on bovine chromosome 7. The *CAPN1* and *CAST* genes, encoding the CAPN1 and CAST enzymes, have been shown to be associated with variation

in meat tenderness (Schenkel *et al.*, 2006; Page *et al.*, 2002). Furthermore, polymorphisms at the *CAST* gene have been found to be correlated with WHC and colour in beef and pork (Reardon *et al.*, 2010; Ciobanu *et al.*, 2004).

1.2.2 Ageing

Ageing is defined as storing meat at refrigerated temperature for a period of time. Ageing at *post mortem* makes important contributions to the increase in beef tenderness and development of flavour characteristics. There are two types of ageing process, wet and dry ageing. Wet ageing, also called vacuum ageing, is widely used in the meat industry due to its high production yield and convenience in storage and transport (Warren & Kastner, 1992). In the dry ageing process, meat is unpacked and exposed directly to environmental conditions. Dry-aged beef is characterised by its good, unique flavour and product quality (DeGeer *et al.*, 2009). However, the dry ageing process is costly because of high ageing shrinkage, trim loss, risk of contamination, and its specific requirements of ageing conditions and space (Parrish *et al.*, 1991).

Traditionally, beef cuts were stored unpacked in a cooler for a period of time to improve tenderness and flavour which is the dry ageing approach (Warren & Kastner, 1992). However, wet ageing was developed in the 1960's due to the trend toward large volume of merchandising and centralized processing and it has become the prevailing ageing method in today's meat industry (Hodges *et al.*, 1974). As a consequence, the traditional dry ageing has become more of a speciality item (Warren & Kastner, 1992). The dry ageing, however, is still a successful process used by some upmarket restaurants and hotels to meet the consumer's preference for the unique product. It also attracts retailers who are looking for ways to generate consumer appeal (Smith *et al.*, 2008).

A new dry ageing process using a highly water vapour-permeable bag (dry ageing bag) has been introduced to the market to improve the traditional unpackaged dry ageing process. Meat aged in dry ageing bags are expected to have the same sensory quality as traditional unpackaged dry aged meat but with less ageing and trim losses, lower risk of contamination and fewer requirements on environmental control (DeGeer *et al.*, 2009; Ahnström *et al.*, 2006).

Studies about the beef ageing process have mainly focused on its effects on beef tenderness and sensory quality. Many consumers are more familiar with wet-aged flavour. Consumers are, however, willing to pay more for dry-aged products and may also prefer the dry-aged flavour when they have become familiar with this type of meat (DeGeer *et al.*, 2009). The studies included in this thesis carried out comparisons of different ageing processes. Moreover,

two different muscles, *longissimus thoracis et lumborum* (LTL) and *gluteus medius* (GM), were used in our studies. The GM is regarded as a good quality muscle in the meat industry, but it is not as good as the *longissimus* muscle. To our knowledge, this is the first time the dry ageing process focused on the GM muscle has been reported.

1.3 Beef quality and grading system

In Europe, carcass quality is assessed through the EUROP system for classification of carcasses. In Sweden, the payment to the farmer is based on the EUROP classification and weight. The highest price per kilogram is paid for young cattle with carcass weights of 250-400 kg, a good carcass conformation and an optimum fat cover, whereas no consideration is paid to the beef quality. On the other hand, the price of meat in the market is influenced by its quality. It is therefore necessary to involve beef quality into the carcass evaluation to let farmers get better paid when they produce meat of higher quality.

Many studies have been done about the effects of genetic markers on meat quality; however, inconsistent results have been reported when the same markers have been tested in different animal populations. No study has ever been done on the Swedish beef population. The studies included in this thesis were carried out on a group of young bulls of the Swedish beef population. Moreover, association analyses on beef colour were performed as only a few studies have been carried out on colour.

2 Aims

The overall aim of this thesis was to investigate the genetic and ageing effects on beef quality. The specific aims of the presented studies were:

- To investigate associations of SNPs at the *DGAT1*, *LEP*, *SCD1*, *CAPN1* and *CAST* genes with colour, marbling, WHC and tenderness in meat from the beef cattle population of young bulls in Sweden. An aim was also to obtain more knowledge of the genetic effects on meat colour. The long-term goal was to increase the attention paid to beef quality in carcass evaluation and increase farmer's payment for high quality beef (Paper I and II).

- To compare beef quality and consumer's preference after ageing beef in dry ageing bags, vacuum and traditional dry ageing. The effects of the three ageing methods were investigated in parallel after ageing the LTL muscle for 8 or 19 days (Paper III). The GM muscle samples were aged in dry ageing bags in comparison with vacuum ageing (Paper IV).

3 Materials and methods

3.1 Experimental design and sample collection

3.1.1 Paper I and II

In total 243 young bulls from five beef breeds were included: Angus (n = 43), Charolais (n = 109), Hereford (n = 35), Limousin (n = 35), and Simmental (n = 21). All animals were raised on Swedish farms and slaughtered in eight Swedish commercial slaughter plants according to standard routines. Conformation and fatness class were assessed according to the EUROP schemes modified to the Swedish system, in which 15-point numerical scales are used (Commission of the European Communities, 2005; Swedish Board of Agriculture, 1998). The EUROP classes were transformed to numerical values for conformation (1 = P^-, poorest, and 15 = E^+, best) and fatness (1 = 1^-, leanest, and 15 = 5^+, fattest). The average conformation score was R^+ (9, standard deviation, SD 2.2), and the average fatness score was 3^- (7, SD 1.3). The carcasses were kept in a chilling room at 4 °C overnight. At cutting, a 15-cm-long piece of the *longissimus thoracis* (LT), starting from the 9^{th} or 10^{th} rib, was removed from one side of each carcass. The samples were packed in vacuum and stored at 4 °C until day 7 *post mortem*.

On day 7 *post mortem*, pH was measured at the anterior side of the meat sample. A 1-cm-thick slice of the LT was cut off from the anterior side of the meat sample to get a fresh surface, where after a 2-cm-thick slice of the LT was cut out for marbling and colour measurements. Subsequently, another 2-cm-thick slice was cut off for additional analyses. For genotyping purposes, a muscle tissue sample was taken from the cross section after the second 2 cm slice, put into 1.5 ml micro tubes and stored at -80 °C until DNA extraction. The remaining part of the LT, at least 7 cm, was packed in vacuum and stored at -20 °C until analyses of WHC and tenderness.

3.1.2 Paper III

Ten steers of Swedish red breed were selected. The animals were on average 24 months old (SD 1.6). All animals were slaughtered at a commercial slaughter plant according to their standard routines. The average carcass weight was 334 kg (SD 52). Conformation and fatness were graded according to the EUROP schemes modified to the Swedish system. The average conformation score was O (5, SD 0.9) and the average fatness score was 3 (8, SD 0.8). The carcasses were stored at 4 °C for 48 h. On day 2 *post mortem*, the muscle LTL was cut out from both sides of each carcass from the 11^{th} rib to the last lumbar vertebrae (around 55 cm). To make sure that all samples had a pH lower than 5.8, muscle pH was measured on the cross section of LTL at the anterior side. For each pair of LTL muscles from the same animal, the first 5 cm from the anterior side was cut off as a control, the remaining parts were cut into six pieces (each was 16 cm long) and assigned to three alternative ageing methods (dry ageing bag, traditional dry ageing, vacuum) for 8 or 19 days, i.e. in total 10 or 21 days *post mortem*. The six treatments were distributed along the two LTL muscles from the same animal according to an extended Latin Square design with randomized order of location on the first animal.

After each ageing time and trimming, pH was measured on one cross section of each piece, colour was measured and samples for water content and sensory panel evaluation were taken and stored at -20 °C in vacuum until analysis.

Meat samples from another two heifers of beef crossbreed were collected for consumer test. The animals were 22 and 25 months old with carcass weights of 315 and 344 kg respectively. The conformation scores were R^+ and R and the fatness score was 3^+ for both. The muscle LTL was cut out from both sides of each carcass from the 11^{th} rib to the last lumbar vertebrae (around 50 cm). On day 7 *post mortem*, the two LTLs from the same animal were randomly assigned into dry ageing bag or vacuum and aged for 21 days, i.e. in total 28 days *post mortem*.

3.1.3 Paper IV

Eight heifers (22-24 months old) of the Hereford breed were used in this study. All animals were slaughtered on the same day at a commercial slaughter plant according to their standard routines. The average carcass weight was 240 kg (SD 32). Conformation and fatness were graded according to the EUROP schemes modified to the Swedish system. The conformation score was P^- and the fatness score was 5^+ for all heifers. On day 6 *post mortem*, the muscle GM was cut out from both sides of each carcass and vacuum packed. On day 7 *post mortem*, muscle pH was measured at the anterior side of GM. The left and right

side muscles from the same animal were assigned to dry ageing bag or vacuum according to a randomised order on the first animal and then aged for 14 days, i.e. in total 21days *post mortem*.

After ageing and trimming, pH was measured at the anterior side of each GM muscle, colour was measured and samples for water content, shear force and consumer test were taken, packed in vacuum and stored at -20 °C until analysis.

3.2 Ageing

In Paper III and IV, three ageing processes were included. (1) Dry ageing bag (Paper III and IV). The bags were made of a polyamide mix and were 50 μm thick with water vapour transmission rate (g/50 μ/m^2/24 h, 38 °C, 50% relative humidity) of 5000. (2) Traditional dry ageing (Paper III). Samples were aged in the cooling room without any package. (3) Vacuum (Paper III and IV). The vacuum bags were 68 μm thick, the permeability (cm^3/m^2, 24 h, 23 °C, 0% relative humidity) were 20 for O_2 and 100 for CO_2. During the ageing process, all samples were kept on stainless steel gratings in darkness in a cooling room which had an average temperature of 2.9 °C and an average humidity of 91%. Samples were turned over and rotated among shelf positions every day to minimise location effects. After the ageing process, samples were unpacked and a smell score of the raw meat was decided by three trained individuals together immediately after opening the package. The evaluation scale indicated how much the odour deviated from that considered to be normal for this kind of meat. The scale was divided in 5 categories from 1 (non off-odour) to 5 (strong off-odour). The weight losses were calculated as follows.

$$\text{Ageing loss (\%)} = \frac{\text{weight before ageing} - \text{weight after ageing}}{\text{weight before ageing}} \times 100\%$$

$$\text{Trim loss (\%)} = \frac{\text{weight before trimming} - \text{weight after trimming}}{\text{weight before trimming}} \times 100\%$$

$$\text{Total ageing and trim loss (\%)} = \frac{\text{weight before ageing} - \text{weight after trimming}}{\text{weight before ageing}} \times 100\%$$

3.3 Analysis methods

3.3.1 pH

The pH (Paper I-IV) was measured on samples used in all studies with a portable pH meter (Knick Portamess® 913, Berlin, Germany) equipped with a combination pH gel electrode (SE 104, Knick, Berlin, Germany).

3.3.2 Colour

Samples for colour measurement (Paper I, III and IV) were wrapped with oxygen-permeable PVC film (NORM PACK 115 45-1, Tempac AB, Tyresö, Sweden) and stored at 4 °C in darkness. Colour was measured on a newly cut surface through the PVC film after blooming for 1.5 h and then after respective storage time in each study. The meat colour was measured using a Minolta CM-2500d/600d spectrophotometer (Konica Minolta Sensing Inc., Osaka, Japan) with 8 mm diameter measuring aperture, illuminant D65, 10° standard observer and CIE L*, a*, b* colour scale. The measuring aperture was covered with a glass plate and the instrument was calibrated against a white plate. The average of four measurements on the meat surface was used. The Minolta instrument recorded reflectance values in the range of 360 nm to 740 nm with 10-nm intervals. Reflectance values that were not directly given by the colour instrument at specific wavelengths (474, 525 and 572 nm) were calculated according to linear interpolation. Then, the Kubelka-Munk K/S values were calculated. The relative content of DeoxyMb was calculated as (K/S474)/(K/S525), the relative content of OxyMb as (K/S610)/(K/S525) and the relative content of MetMb as (K/S572)/(K/S525) (AMSA, 2012). Because the K/S ratio decreases when the relative content of the corresponding myoglobin form increases, the K/S ratios were transformed to make the changes in the myoglobin species easier to interpret (Lagerstedt *et al.*, 2011). The K/S ratio of DeoxyMb was transformed to [1.5 - (K/S474)/(K/S525)], the K/S ratio of OxyMb was transformed to [1 - (K/S610)/(K/S525)] and the K/S ratio of MetMb was transformed to [2 - (K/S572)/(K/S525)]. The chroma value was calculated as $(a^{*2} + b^{*2})^{1/2}$ and the hue angle as arctan b*/a*.

3.3.3 Marbling

The samples for marbling analysis (Paper I) were photographed on both sides using a digital camera (Panasonic DMC-FZ50, Osaka, Japan). A green paper was used as background to strengthen contrast and eliminate confusion about the boundaries between the meat sample and the background. To avoid reflection from the surface of the muscle, a polarizing filter (HOYA, Circular Polarizing, Tokina Co., Ltd., Japan) was used on the camera together with

polarized cover on the photo lamps. Muscle samples were also dabbed by soft tissue paper before taking photos to avoid reflection. These photos were saved as RAW and JPEG files for further image analysis.

Image analysis

Sample images of RAW files were manually set to strongest contrast (2.50) and saved as TIF files with the image development software (SILKYPIX Developer Studio 3.0 SE, Copyright 2004-2009, Ichikawa Soft Laboratory, Chiba, Japan). The TIF files were then imported to a photo editing software (Adobe® Photoshop® Elements 8.0, Copyright 1990-2009, Adobe Systems Incorporated, San Jose, California, USA) to manually remove all visible fat and connective tissue surrounding the LT muscle on the meat sample images. The TIF files were finally imported to image analysis software (Assess 2.2, Copyright 2008, The American Phytopathological Society, Saint Paul, Minnesota, USA) to evaluate intramuscular fat content. The colour space HSI (hue, saturation and intensity) was used. The hue thresholds were set with an upper value of 80 and a lower value of 25 to separate the meat from the background, i.e. select meat as the area of interest. The saturation values were set between 0 and 80 to determine the number of white pixels (intramuscular fat) in the area of interest. The percentage of white pixels in the area of interest was used as an estimate of intramuscular fat content in the meat sample (Garcia *et al.*, 2006).

Subjective marbling score

Visual marbling scores on both sides of each meat sample were assessed using the JPEG files by two trained panellists individually. Five photographs with different level of intramuscular fat deposition were selected as reference. Assigned marbling scores were from 1 to 5 with 0.5 intervals (1 = no visible intramuscular fat, 5 = medium amount of intramuscular fat as no individual with high level of marbling was found within the present group of young bulls).

3.3.4 Water holding capacity

The frozen samples (Paper I and IV) were thawed at 4 °C overnight and then put into a room temperature water bath for 30 min to equalize the sample temperature. The samples were weighed, repacked in vacuum and then heat treated in a 72 °C water bath until a core temperature of 70 °C. The samples were cooled in running cold tap water for 30 min, then stored at 4 °C overnight and weighed the next day. Water holding capacity was calculated as follows.

$$\text{Thawing loss (\%)} = \frac{\text{weight before freezing} - \text{weight after thawing}}{\text{weight before freezing}} \times 100\%$$

$$\text{Cooking loss (\%)} = \frac{\text{weight before cooking} - \text{weight after cooking}}{\text{weight before cooking}} \times 100\%$$

$$\text{Total loss at thawing and cooking (\%)} = \frac{\text{weight before freezing} - \text{weight after cooking}}{\text{weight before freezing}} \times 100\%$$

3.3.5 Instrumental tenderness

The instrumental tenderness was measured using a Stable Micro Systems Texture Analyser HD 100 (Godalming, UK). Samples were cooked as described above and then stored at 4 °C overnight. For each sample, 3-cm-long strips were cut out with 10 mm × 10 mm cross-sectional area and the direction of muscle fibres parallel to the longitudinal dimension of the strips.

Warner-Bratzler shear force

The Warner-Bratzler shear force (Paper II and IV) was measured using the method described by Honikel (1998). In this measurement, the instrument was equipped with a blade with a rectangular shaped cutting area of 11 mm × 15 mm. The blade was 1 mm thick and had the speed of 0.83 mm/s when cutting through the strips. Twelve strips from each sample were measured and peak force (N), total energy (Nmm) and shear firmness (N/mm) were recorded.

Compression

The compression (Paper II) was measured using the method described by Honikel (1998). Compression measurements were conducted by a squared flat ended plunger (10 mm × 10 mm), which was driven with test speed of 1.60 mm/s vertically 80% of the way through the strips. The direction of muscle fibre was perpendicular to the direction of plunger penetration and the plunger was driven twice into the strips at the same point. The strips were placed in a metal cell (50 mm long × 10 mm wide × 20 mm high) with two lateral walls parallel to the longitudinal direction of the strips. During compression test, the resulting deformation of strips only happened in the direction parallel to the muscle fibre. Compression results were recorded as hardness (N) and compression energy (Nmm).

3.3.6 Water content

Water content (Paper III and IV) were analysed separately between the 2 cm outer layer and the next 2 cm inner layer. Duplicate samples of 3 g meat were chopped into small pieces and then put into oven-dried aluminium tins. The aluminium tins with the sample were kept in oven at 105 °C for 16 h and then cooled in desiccator for 1 h. The water content was calculated as weight loss during drying in the oven in percent of sample weight before drying.

3.3.7 Microbiological analyses

Samples for microbiological analyses were collected before ageing as control (Paper III and IV), after ageing but before trimming (Paper III and IV) and after trimming (Paper IV). Four 2-mm-thick samples with a 2.5-cm diameter were aseptically taken from each sample. In Paper III, samples were taken from each dorsal subcutaneous fat and ventral lean side separately.

The samples were put into a blender bag (Grade Packaging, VWR), mixed with 100 ml of buffered peptone water and stomached in a blender (easyMIX®, AES Laboratoire, France) for 2 min. Appropriate serial decimal dilutions of the homogenate were made in peptone saline (0.1% peptone in 0.85% NaCl) and 0.1 ml of each dilution was plated on respective agars and incubated (Table 3). LAB counts were incubated in an anaerobic jar with a disposable anaerobic conditions generator (AnaeroGen, Oxoid). To confirm that colonies growing were EB, the oxidase test (Becton, Dickinson & Co.) was performed.

Table 3. *Agar and incubation condition used for microbial growth (Paper III and IV)*

Microbial	Agar	Incubation condition
Total bacteria counts	Plate Count Agar	30 °C for 72 h, aerobic
Enterobacteriaceae	Violet Red Bile Glucose Agar	37 °C for 24 h, aerobic
Lactic acid bacteria	de Man, Rogosa and Sharpe Agar	25 °C for 5 days, anaerobic
Yeast and mould	Sabouraud Dextrose Agar with Chloramphenicol	25 °C for 7 days, aerobic

3.3.8 Sensory analyses

Sensory panel evaluation

The sensory test was carried out at the University of Copenhagen, Denmark. The panel consisted of 10 selected and trained sensory assessors (6 women and 4 men; aged 22 to 34 years with an average of 25.7 years). They were chosen for their ability to recognize and discriminate basic tastes according to ISO 3972: 1991 and their ability to detect, discriminate and describe a range of odours. The panel was trained for 4 days prior to the sample evaluation. The

training included the development of a descriptive sensory vocabulary and the discrimination of the samples on the sensory attributes using a 15-cm continuous line scale. The scale anchors were from “none” or “a little” to “a lot”. During the development of the sensory vocabulary, reference standards were used to clarify the attributes for the panel members. The sensory attributes used in the sample evaluation are listed in Table 4. The sensory evaluations were performed in a test room designed according to ISO 8539: 2007. Each assessor evaluated the samples in individual booths without any disturbances from the surroundings. The room was kept constant at 22 °C and odour free by air extraction. All evaluations were made under artificial daylight (Philips TLD 18w/17).

Table 4. *Description of sensory attributes involved in the sensory test*

Sensory attribute	Description
Animal	A smell/taste/after taste of Serrano ham
Metallic	A smell/taste/after taste of iron sulphate
Liver	A smell/taste/after taste of fresh liver
Boiled meat	A smell/taste of boiled meat
Bouillon	A smell/taste of beef broth
Butter fried meat	A smell/taste/after taste of beef shortly fried in butter
Fatty	A taste of fat from beef
Raw	A taste of raw meat
Umami	A taste/after taste of monosodium glutamate (Reference for taste: 0.7 g/L MSG monohydrate)
Salty	A taste of sodium chloride (Reference for taste: 1.4 g/L NaCl)
Sour	A taste/after taste of citric acid (Reference for taste: 0.6 g/L Citric acid)
Sweet	A taste of sucrose (Reference for taste: 7.2 g/L Sucrose)
Bitter	A taste/after taste of quinine (Reference for taste: 0.0036 g/L Quinine.HCl dihydrate)
Tenderness	The degree of tenderness
Juiciness	The degree of juiciness
Crumbly	The degree of crumbliness when the sample is ready to be swallowed
Sticky	The degree of stickiness to the teeth
Fibrous	The degree of fibre feel when the sample is ready to be swallowed
Astringent	The degree of astringency
Coating	The degree of mouth coating when the sample has left the mouth

All the samples used as references for developing sensory attribute vocabulary were traditional dry-aged meat.

The frozen samples were thawed overnight at 2 °C (still slightly frozen) and then sliced into cubes 15 mm thick (7.8 ± 0.3 g) using a ruler and sharp knife. The samples were subsequently cooked in an oven at 195 °C for 5 min in order

to achieve a sample core temperature of 65-68 °C. Samples were served on warm plates (heated in oven at 60 °C) and covered with aluminum foil to retain heat. The evaluation was organized so that each judge had a minimum of 5 min to judge each sample. The data were collected on PC using FIZZ Network Acquisition (Version 2.40E). The samples were evaluated in duplicate and the whole evaluation process lasted for 7 days (2 h per day).

Consumer test

The frozen samples were thawed in refrigerator for 24 h and then cooked in oven at 150 °C until the central temperature reached 65 °C, resulting in a final temperature of 68 °C. Samples were cooled in refrigerator overnight and then cut into 3-mm-thick slices with all edges trimmed off to get a uniform appearance.

The consumer test (Paper III and IV) was carried out at a local supermarket. The cooked beef samples from the same animal and anatomical location were put on a paper plate and labelled by three digit numbers. To minimise the effects of tasting order, equal number of plates with opposite sample order was prepared. The consumers participating in the test were asked to taste the two samples starting from the left side of the plate. After tasting, they had to answer the following questions on the questionnaire: (1) Which sample do you like more? (2) Which sample do you think is more tender? (3) Which sample do you think is juicier? There was no option of "no difference between samples" on the questionnaire but the consumers could write their comments. In addition to the taste test, the participants answered general questions about gender, age and frequency of beef consumption.

3.3.9 Genotyping

DNA was isolated from muscle tissue using the Tissue DNA Kit (E.Z.N.A.™, Omega Bio-Tek Inc., USA) according to the manufacturer's instruction. Allelic discrimination was carried out using Real-Time PCR system (StepOnePlus™, Life Technologies Corporation, USA) with TaqMan chemistry and probes. The polymorphisms in five candidate genes were genotyped (Table 5).

Table 5. *Primer and probe sequences (from 5' to 3') used for genotyping the different polymorphisms*

	Gene				
	DGAT1	*LEP*	*SCD1*	*CAPN1*	*CAST*
SNP name	DGAT1 K232A	UASMS2	SCD1.878	CAPN1:c.947	CAST:c.155
Allele	AA > GC	C > T	G > A	G > C	C > T
Translation	K232A	Promoter	A293V	G316A	P52L
Forward primer	CGCTTGC TCGTAGC TTTGG	AGGTGCC CAGGGAC TCA	CCCCGAGA GAATATTC TGGTTTCC	GGCTGGGC AGGTCAGT	AACAAGCC TTGGGAGC AGT
Reverse primer	CGCGGTA GGTCAG GTTGTC	CAACAAA GGCCGTG TGACA	CCACTAGA CGTGGTCT TGCT	AGCTGCTC CCGCATGT AAG	AAAGTAGG TGTCTTTTC ATGTCCA
VIC probe	CGTTGGC CTTCTTA C	CAAGCTC TAGAGCC TGTGT	CTTACCCG CAGCTCC	CCACGGCG TTCCA	AAAAAGCC CCGGTCC
FAM probe	TTGGCCG CCTTAC	AAGCTCT AGAGCCT ATGT	ACTTACCC ACAGCTCC	CCACGCCG TTCCA	AAAAAGCC CTGGTCC
GenBank ID	AY065621	AB070368	AY241932.1	AF252504	NM_174003.2
Paper	I	I and II	I	I and II	I and II

3.4 Statistical analyses

The statistical analyses were carried out with Statistical Analysis System (Version 9.2, SAS Institute, Cary, NC, USA). In all studies, the procedure MIXED was applied to calculate least-squares means (LSM) and standard error. Degree of freedom was estimated by the Satterthwaite's method. The option PDIFF was used for calculating significant differences between LSM. Procedures used in each paper are summarized in Table 6. The consumer test results (Paper III and IV) were analysed using Chi-square test. In Paper III, the structure in the sensory data was analysed by Principal Components Analysis (PCA) using the data averaged over the animal and sensory replicates. The

PCA model was based on the mean centred, auto-scaled data using full cross-validation (Unscrambler X 10.1, Camo, Norway).

Table 6. *Summary of statistical models have been used in the MIXED procedures*

Response variable	Fixed effect	Random effect	Statement/option	Paper
Colour, marbling, WHC, pH	SNP in five genes, breed, age within breed as a covariate	Slaughter plant within breed	REPEATED with breed as "group"	I
Peak force, shear firmness, total energy, hardness, compression energy	SNP in three genes, breed, age within breed as a covariate	Slaughter plant within breed	REPEATED with breed as "group", Bonferroni correction	II
pH, smell score, weight losses, colour, microbial counts	Ageing method, ageing time and their interaction	Animal		III
Water content	Ageing method, ageing time, sample layer and their interaction	Animal		III
Sensory panel evaluation results	Ageing method, ageing time and their interaction	Animal, assessor		III
pH, smell score, weight losses, WHC, shear force, colour, microbial counts	Ageing method	Animal		IV
Water content	Ageing method, sample layer and their interaction	Animal		IV

4 Results

4.1 Paper I and II

The allele and genotype frequencies were calculated on all 243 animals and the frequencies of all five polymorphisms in each breed were calculated respectively.

In the statistical analysis, individuals with a pH value in meat higher than 5.8 were excluded. Genotype groups within each breed with less than three animals were excluded in the association analysis. Moreover, in Paper II, samples from the wrong part of the LT muscle were also discarded. The remaining number of samples from the Simmental breed was only ten and data from this breed were excluded in the statistical analyses of marker associations with tenderness.

The polymorphisms of the *LEP*, *SCD1* and *CAPN1* genes showed associations with variation of colour parameters after 6 days in air. Higher chroma value and lower relative content of DeoxyMb were observed in meat from individuals with SNP of the *LEP* gene including the C allele (CC and CT) than those carrying the TT genotype on day 6 of exposure to air. Animals with GA and AA genotypes at polymorphism site of *SCD1* gene had higher a*, b*, chroma value and OxyMb relative content in meat on day 6 than those carrying the GG genotype. The hue angle was higher in meat from *CAPN1* gene heterozygotes compared to homozygous GG animals whereas the heterozygotes did not differ from animals carrying the CC genotype.

The phenotypic correlation between IMF content and subjective marbling score was 0.77 ($P < 0.05$). The polymorphisms of the *DGAT1* and the *CAPN1* gene were associated with IMF content and subjective marbling score. Animals with the heterozygous *DGAT1* genotype (AAGC) displayed a higher IMF content and subjective marbling score than those with the homozygous GCGC genotype. For the *CAPN1* SNP, higher IMF content and subjective marbling

score were found in animals carrying the CC genotype than those with CG and GG genotypes.

The SNP in *CAPN1* gene showed a significant effect on shear firmness and compression energy. Meat from animals with the CC genotype was more tender than from those with the GG genotype. Meat tenderness of the heterozygote was intermediate to both homozygotes. Lower peak force, shear firmness, total energy, hardness, and compression energy values were observed for the genotypes including the T allele in the *CAST* gene SNP. The effects of the CT and TT genotypes were not different. The SNP within the *LEP* gene showed association with hardness and compression energy; however, no genotype differences remained after Bonferroni adjustments.

There was no significant association between any tested SNPs and meat colour traits on day 0 in air, nor was there any association with WHC and variation in pH value.

4.2 Paper III

The smell score of the raw meat when opening the package was lower for samples in dry ageing bags than those in vacuum after ageing for 8 and 19 days, and it was also lower than samples aged by traditional dry ageing for 19 days. Higher smell score (stronger off-odour) was observed in samples after traditional dry ageing for 19 days compared with 8 days dry ageing.

The ageing loss of meat aged in dry ageing bags was lower than after traditional dry ageing for 8 and 19 days but it was higher than that after ageing in vacuum. The trim losses after ageing for 19 days and total ageing and trim loss after both ageing times were higher after both dry ageing methods than after vacuum ageing. The weight losses increased with ageing time after using dry ageing methods.

The water content of meat samples before ageing was on average 73.9%. The water content of the outer layer for samples aged in vacuum was higher than those aged using traditional dry ageing after both 8 and 19 days, and it was also higher than samples aged in dry ageing bags for 19 days. The water content of the inner layer for samples aged in vacuum was higher than those aged using traditional dry ageing for 19 days. The water content of samples after traditional dry ageing (both outer and inner layers) and the outer layer of samples in dry ageing bags decreased with longer ageing time. The water content differed between layers after ageing using both dry ageing methods for 19 days.

There was no effect of ageing method on beef colour. Only a tendency of higher a* value, chroma and relative content of OxyMb were found in samples

aged in vacuum than using traditional dry ageing after ageing for 19 days. The L* value of samples aged in dry ageing bags and the hue angle of samples aged in all ageing methods increased with longer ageing time ($P < 0.05$).

The TBC, EB and yeast counts were lower on the meat side of samples aged in dry ageing bags than after traditional dry ageing, but higher than in vacuum for both ageing times. The TBC on the fat side of samples using traditional dry ageing were higher than those in vacuum after both ageing times. The LAB counts on the fat side of samples using dry ageing methods were lower than those in vacuum after both ageing times. The yeast counts on the fat side of samples after using dry ageing methods were higher than those in vacuum for both ageing times. There was no effect of ageing method or ageing time on mould counts.

The sensory panel detected no differences for most of the sensory attributes between samples using the two dry ageing methods, except for the odour of the cutting surface, e.g. metallic, liver and butter fried meat. Higher umami and butter fried meat taste were found in samples aged using dry ageing methods than packed in vacuum and also higher tenderness after 19 days. No effects of ageing method and ageing time were found on the odour of bouillon on the cutting surface, raw taste, the flavour of umami, sour and bitter after taste, sticky texture and mouth sensation of astringent and coating. The PCA of the sensory profiling data showed a clear separation between the ageing times within all of the ageing methods. Smaller differences were observed between the ageing methods. PC1 (55%) mainly described the variation between the ageing times, whereas both PC1 and PC2 (17%) showed differences between traditional dry ageing and the two bagging methods. The attributes of umami, bouillon and salty were grouped along PC1 and showed some differences in the way they were evaluated as odour, flavour or aftertaste, respectively. The animal after taste, butter fried meat, fatty, tenderness and juiciness were also grouped together with these attributes. Boiled meat and metallic flavour as well as fibrous mouth feeling were correlated with PC1 in the opposite direction. PC2 related to the attributes of liver odour and to a lesser extent metallic and animal odour.

For the consumer test, the *longissimus* muscle aged during 21 days in a dry ageing bag was compared with meat from the other side of the same animal aged the same time in vacuum. In total 264 participants took part (42.0% females and 58.0% males) and almost half of them (48.1%) were between 41-65 years old. A majority of the participants consumed beef at least once a week. If a consumer found no difference for one of the attributes between samples aged by the two ageing methods, this answer was excluded from the statistical analysis. Of the consumers that showed preferences on attributes,

60% of the consumers preferred meat aged in dry ageing bags; 67% considered that meat as being more tender and 64% found it juicier than vacuum-aged meat ($P < 0.05$).

4.3 Paper IV

The ageing method had effects on weight losses, WHC, water content and microbial counts. No differences were found in pH value, smell score of the raw meat, shear force and meat colour between samples aged using different ageing methods. Higher ageing and trim losses but lower thawing loss, cooking loss and water content were found in samples aged in dry ageing bags compared to those aged in vacuum. Samples aged in dry ageing bags had higher total bacteria and yeast counts but lower LAB counts than those aged in vacuum, both before and after trimming. No differences were found in EB and mould counts between samples using different ageing methods.

For the consumer test, in total 129 participants took part in and made 1 or 2 independent tastings and the total number of answers was 169. A major part of the participants (42.6%) were between 41-65 years old and most of them (42.6%) said they consumed beef at least once a month. Meat aged in dry ageing bag was more tender and juicier and overall preferred by consumers compared with samples aged in vacuum. Females and males behaved differently in their responses. Females preferred meat aged in the dry ageing bag, and also found it more tender and juicier than meat aged in vacuum, whereas the males found no difference between samples using different ageing methods.

5 Discussion

5.1 Association analysis of SNPs and beef quality

5.1.1 Colour

In our study, associations between SNPs at the *LEP*, *SCD1* and *CAPN1* genes and variation in meat colour were found (Paper I). The heritability for myoglobin concentration was found to be 0.85 in beef on day 0 of retail display and for beef colour parameters after 6 days display the heritabilities varied between 0.13 and 0.41 (King et al., 2010). Thus, there is a considerable genetic influence on retaining meat colour.

The *LEP* and *SCD1* are genes that are related to meat fat deposition and metabolism. The intramuscular fat content has been suggested to influence the visual appraisal of meat colour (Fiems *et al.*, 2000). Moreover, high fat content accelerates muscle lipid oxidation (Ahn *et al.*, 1998). Myoglobin oxidation, which leads to meat discoloration, is linked to lipid oxidation. The myoglobin and lipid oxidation can exacerbate each other through the chemical compounds formed in the oxidation process (Faustman *et al.*, 2010). In our study, the observed correlations of IMF content and subjective marbling score with a*, chroma, hue angle, relative content of DeoxyMb, OxyMb and MetMb on day 6 ($P < 0.05$, data not shown) support the previously suggested connection between marbling and both the objective measures and visual perception of meat colour. Also the observed associations of the *LEP* and *SCD1* polymorphisms with meat colour traits were quite consistent with previously observed connection between marbling and meat colour. One hypothesis of the possible connection between the *SCD1* gene and meat colour may be through reduced nicotinamide adenine dinucleotide (NADH), NADH-cytochrome b_5 reductase, and cytochrome b_5. When the enzyme SCD1 catalyzes the biosynthesis of MUFAs, NADH provides reducing equivalents, NADH-cytochrome b_5 reductase acts as flavoprotein and cytochrome b_5 as electron

carrier (Ntambi, 1999). Moreover, they also play important roles in the MetMb reducing process: NADH acts as a coenzyme, where NADH-cytochrome *b5* reductase is the main enzyme in this system and transfers two electrons from NADH to two molecules of cytochrome *b5* (Shirabe *et al.*, 1992). MetMb receives the electrons from reduced cytochrome *b5* and is reduced to DeoxyMb (Bekhit & Faustman, 2005). However, this hypothesis still needs to be confirmed by other studies.

Reardon *et al.* (2010) observed an association of a marker at the *SCD1* gene (SCD1.702) with beef colour on day 2 *post mortem*. Animals with AA genotype had lower a* value than animals with GG. In our study, individuals with GG genotype in the SNP at the *SCD1* gene (SCD1.878) had a lower a* value than animals with the alternative genotypes. Both the tested SNPs, SCD1.702 and SCD1.878, are located in the open reading frame of the *SCD1* cDNA. The SNP of SCD1.878 causes a substitution of the amino acid valine to alanine in the amino acid position 293 whereas the SCD1.702 polymorphism is a synonymous mutation. The A allele of SCD1.702 and the G allele of SCD1.878 are in linkage disequilibrium and represent the allelic variant of the protein with the alanine in position 293 (Taniguchi *et al.*, 2004). Thus, both studies mentioned above found that animals with the amino acid alanine at the SCD1 protein 293 position had less intense red beef colour.

The marker CAPN4751 in the *CAPN1* gene showed dominant effects on colour of meat from Nellore cattle after 7, 14 and 21 days *post mortem* in a study by Pinto *et al.* (2011). Reardon *et al.* (2010) found an association between a marker at the *CAST* gene (CAST257) and beef colour on day 2 *post mortem*. Thus, polymorphisms in genes related to μ-calpain activity might affect meat colour traits, directly or indirectly. This indication finds support in the present study, in which the SNP in the *CAPN1* gene was associated with variation in meat colour measured as hue angle.

5.1.2 Marbling

For the DGAT1 K232A polymorphism, the AA allele codes for the amino acid lysine (K) and GC codes for alanine (A). In our study (Paper I), animals with the heterozygous AAGC genotype (i.e. with amino acids K and A) had both a higher IMF content and degree of subjective marbling compared with the GCGC genotype (i.e. homozygous for the amino acid alanine). This is in accordance with former reports in which the allele encoding lysine has been associated with higher milk fat and IMF content (Grisart *et al.*, 2004; Thaller *et al.*, 2003; Winter *et al.*, 2002). This has been suggested to be due to the alanine residue at position 232 of the DGAT1 protein having a negative effect on the

acyl-CoA binding ability and thereby resulting in a less efficient fat synthesis (Winter *et al.*, 2002).

The correlation between the two measures of marbling, IMF content and subjective marbling score, was not higher than 0.77 in our study. This might be due to the very low degree of marbling of the meat samples. Fat deposed as small white dots might not have been visible to the naked eye, but could be recognized by the applied image analysis program. Even though image analysis is a more objective measure of marbling and therefore a better indicator of the final eating experience, visible fat is what the consumers base their purchase decision on in the store. Thus, both measures are of relevance for consumer acceptance.

5.1.3 Tenderness

The most common allele, G, in the CAPN1:c.947 SNP, which is included with the name T2 in the Gene STAR test (Johnston & Graser, 2010), has an unfavourable effect on meat tenderness. The CAPN1:c.947 polymorphism results in an amino acid substitution from alanine to glycine (Barendse *et al.*, 2007).

In our study, the T allele in the CAST:c.155 SNP was the most common in all breeds except in Simmental. The results indicated that the T allele has a favourable effect on tenderness, which is in accordance with the study by Barendse *et al.* (2007). These results indicate that this SNP is a useful marker for predicting tenderness in meat from young bulls.

Our results do not provide any strong evidence for an association between the UASMS2 SNP and tenderness, nor do they support the results by Gill *et al.* (2009) who found that meat from animals with the T allele was the most tender and that the C allele showed a dominant effect. The UASMS2 SNP was selected in our study as one of the analysed markers because of its association with intramuscular fat (Nkrumah *et al.*, 2005) and because marbling has been suggested to influence tenderness (Thompson, 2004). However, our results indicate that the UASMS2 SNP is not useful for predicting tenderness in Swedish beef of young bulls. These results are in agreement with the study by Schenkel *et al.* (2005) who showed that the UASMS2 SNP was not associated with tenderness.

5.1.4 Water holding capacity

In our study, no association between the analysed SNPs and WHC traits was found. However, another study found that polymorphisms in the *CAST* and *SCD1* genes were associated with pH value at 48 h *post mortem* which they used as a measure of WHC (Reardon *et al.*, 2010). The reason for the lack of

corresponding result in our study may be due to the freezing of the samples used for WHC measurements as the WHC becomes lower after freezing (Lagerstedt *et al.*, 2008).

5.2 Effects of ageing on beef quality

5.2.1 Raw meat smell score

The smell immediately after opening a package of meat is of great importance as it is the first impression, apart from the visual impression, a consumer gets of the meat product. In our studies, both the sensory panel and the consumers tasted and described sensory characteristics of cooked meat. Thus, the smell score of the raw meat improved the description of the sensory quality of meat. In Paper III, the smell score of the raw meat after the ageing process indicates that the samples aged in dry ageing bags for 19 days had less off-odour than those aged by traditional dry ageing and that the smell of samples aged in dry ageing bags did not deteriorate with prolonged ageing time.

5.2.2 Weight losses, water content and water holding capacity

It was expected that the ageing and trim losses would be higher for steaks aged using dry ageing methods than in vacuum, since dry aged meat is known to lose more moisture during the ageing process and that the resulting dry surface layer has to be trimmed off. It is of interest to investigate if there are differences in weight losses between samples aged using dry ageing bag compared with traditional dry ageing. A lower ageing loss was found in samples aged in dry ageing bags compared with traditional dry ageing (Paper III). Ahnström *et al.* (2006) also reported similar results, but only after ageing for 21 days. The trim loss and total ageing and trim loss were not different between samples using the two dry ageing methods, which were in accordance with the result from DeGeer *et al.* (2009), whereas Ahnström *et al.* (2006) found higher trim loss in steaks using traditional dry ageing than in dry ageing bags after ageing for 21 days. It is, however, difficult to standardize the degree of trimming after dry ageing which may be the reason for the somewhat inconsistent findings.

In accordance with the ageing loss results, it was not surprising that the water content of samples aged using dry ageing methods was lower than that of vacuum-aged meat. The emphases in our studies were the magnitude of the difference between dry and wet ageing and between the two dry ageing methods. In the study using GM muscle, even the water content of the 2-cm inner layer of meat aged in the dry ageing bags was lower than that of vacuum aged meat (Paper IV). The water content of the outer layer differed between

traditional dry ageing and vacuum regardless of ageing time, whereas a difference was only observed between samples aged in dry ageing bags and vacuum after ageing for 19 days. No difference was observed on the water content of the inner layer between samples aged in dry ageing bags and vacuum after ageing for 19 days. Moreover, only samples using traditional dry ageing showed decreased water content at the inner layer with longer ageing time (Paper III). All these results indicate that the water vapour rate slowed down in dry ageing bags compared with traditional dry ageing.

The WHC was higher in meat aged in dry ageing bags than in vacuum (Paper IV). Laster *et al.* (2008) found that the cook yield was higher for dry-aged top loin and top sirloin steaks than wet-aged steaks. Although the study by Warren and Kastner (1992) did not find a significant difference in cooking loss between dry-aged and vacuum-aged strip loins, the mean values for dry-aged meat were lower than for vacuum-aged counterparts. However, our results showed that the total loss at ageing, thawing and cooking was still higher in meat aged in dry ageing bag than in vacuum (Paper IV), which means that the higher WHC in meat aged in dry ageing bags cannot compensate for the weight loss during ageing and trimming. Lower product yield in dry-aged meat exists also after using dry ageing bags, compared with the vacuum ageing process. Lower total saleable yield was also observed for traditional dry-aged steaks compared to wet-aged steaks (Laster *et al.*, 2008; Smith *et al.*, 2008).

5.2.3 Colour

The meat colour after different ageing processes has, to our knowledge, not been measured in earlier studies. The results from our studies indicate that there was no significant effect of ageing method and ageing time on meat colour. Ageing meat in dry ageing bags had no negative effects on meat colour compared to vacuum-aged meat (Paper IV). The meat colour was slightly redder for samples in dry ageing bags than using traditional dry ageing (Paper III). Because myoglobin oxidation leads to meat discoloration, this indicates that less myoglobin oxidation occurred in samples aged in dry ageing bags than using traditional dry ageing. In the study by DeGeer *et al.* (2009), they found less lipid oxidation occurring in steaks aged in dry ageing bags than using traditional dry ageing. Myoglobin oxidation and lipid oxidation can accelerate each other through the chemical compounds formed during oxidation process (Faustman *et al.*, 2010). Thus, the results concerning colour in our study and the lipid oxidation results from DeGeer *et al.* (2009) support each other.

5.2.4 Microbial growth

The microbial counts in general increased for all ageing methods with ageing time. More microbial counts were found in samples aged using dry ageing methods than in vacuum ageing. The dry ageing bag is a relatively new packaging material, where water, air and oxygen can pass, but not microorganisms including virus. These characteristics of dry ageing bags can thus protect the meat from being contaminated, and also modify the growth of microorganisms already present in meat. Samples aged in dry ageing bags could have similarities and differences with both vacuum-aged and traditional dry-aged samples.

The mould and EB counts in samples aged in dry ageing bags were similar to those in vacuum-aged samples, suggesting that no extra growth had occurred in the dry ageing bags as a result of the treatment. LAB counts were lower in dry ageing bags than those in vacuum-aged samples (Paper IV). Similar results were also reported by Parrish *et al.* (1991) and were most likely due to the anaerobic conditions in vacuum packaging that result in the dominance of this type of bacteria, in contrast to meat exposed to aerobic conditions (Ahnström *et al.*, 2006). The TBC and yeast counts were higher in samples aged in dry ageing bags than those aged in vacuum, both before and after trimming. This was possibly a consequence of the transmission of oxygen through the dry ageing bag, and for the yeast, also because they can tolerate the low water activity. Our study showed that some of the microbiological parameters, most importantly the TBC counts, were higher in samples aged in dry ageing bags than in vacuum. However, judging from both smell test results of the raw meat and the moderate levels of the microbiological growth, all the samples were acceptable with regard to food safety.

More differences between samples aged using dry ageing methods were observed on the meat sides compared to the fat sides (Paper III). Lower TBC, EB and yeast counts were found on the meat side of samples aged in dry ageing bags than using traditional dry ageing; in addition, only samples aged using traditional dry ageing had increased mould counts with longer ageing time. These results indicated that it is possible to better control microbial growth with dry ageing bags than with traditional dry ageing. Ahnström *et al.* (2006) found higher yeast counts on the lean side of beef strip loins using traditional dry ageing than in dry ageing bags aged for 14 or 21 days. DeGeer *et al.* (2009) only found higher yeast counts on shell loins using traditional dry ageing but not in dry ageing bags. This means there are advantages of using the dry ageing bag, e.g. to decrease the risk of microbiological contamination compared with traditional unpacked dry ageing. The LAB counts, which are anaerobic, tended to be higher on the meat side of samples aged in dry ageing

bags for 19 days than using traditional dry ageing. This may be due to the structure of the dry ageing bags, as the membrane of the bag could have some barrier function although it is oxygen permeable.

5.2.5 Sensory properties

The sensory panel evaluation results (Paper III) showed that samples aged using dry ageing methods had higher scores on some typical attributes compared to those aged in vacuum, e.g. umami, butter fried meat (i.e. a nutty odour) and tenderness. Generally, small differences in odour and taste attributes between samples using the two dry ageing methods were found. Ahnström *et al.* (2006) found no difference in sensory traits between samples aged in dry ageing bags and traditional dry ageing. DeGeer *et al.* (2009) found differences only in sweet and bitter tastes between samples aged in dry ageing bags and traditional dry ageing. In our study, small increases in the odour of the cutting surface for metallic and liver were observed for samples aged using dry ageing bags, whereas the butter fried meat odour at the cutting surface slightly decreased. The slight increase in metallic odour of the meat aged in dry ageing bags was also noticeable as aftertaste. A similar metallic odour and taste was observed in the vacuum-aged beef. Metallic odour is known to derive from oxidative changes of lipids, e.g. through the formation of volatile compounds such as expoyalkenals. Furthermore, ferrous iron has been shown to be an important source for metallic taste (Ömür-Özbek *et al.*, 2012). Even though the effects in comparison to the traditional dry ageing method were small, the dry ageing bag and vacuum ageing methods may lead to some redox changes resulting in the formation of volatiles with a metallic aroma. This process may be further stimulated after cutting in the presence of an increased oxygen level. Interestingly, the liver odour was also increased after cutting of meat aged in dry ageing bags and vacuum. The volatile aroma compounds responsible for liver odour contain lipid-derived aldehydes and sulphur compounds which further support the notion of oxidative changes. The butter fried meat and fatty flavours were perceived the least intense in the vacuum-aged beef samples. Even though these sensory effects were small, they indicate that the vacuum ageing method may give more protection against fat oxidation. Further evidence for oxidative changes were found in the effects of ageing time on the meat sensory attributes, where the liver and butter fried meat attributes significantly increased when the meat samples were aged for 19 days compared to 8 days. The sensory tests indicate that beef samples aged in dry ageing bags have similar odour of cutting surface and aftertaste to the vacuum-aged samples, but provide an improved umami flavour and tenderness which are close to the traditional dry-aged samples. The ageing process carried out using

dry ageing bags is a combination of traditional dry ageing and vacuum ageing that develop characteristics both from traditional dry ageing and vacuum ageing.

The consumer tests showed that the consumers were able to indicate overall preferences, tenderness and juiciness between beef aged in dry ageing bags and vacuum. Meat aged in dry ageing bags was mostly preferred by consumers using both LTL and GM muscles, and they considered the samples aged in dry ageing bags being more tender and juicier (Paper III and IV).

5.2.6 Effects of ageing time

Ageing time had effect on most of the beef quality traits involved in our study (Paper III), e.g. umami and tenderness of the samples increased with longer ageing time; although weight losses and microbiological counts also increased. Other studies also found increased weight losses with extended ageing time, whereas the flavour was not significantly increased with longer ageing time (DeGeer *et al.*, 2009; Ahnström *et al.*, 2006) or consumers could not detect flavour differences between ageing periods (Laster *et al.*, 2008; Smith *et al.*, 2008). However, the consumers overall likeness and the tenderness and juiciness of the dry-aged meat increased as ageing time increased (Laster *et al.*, 2008; Campbell *et al.*, 2001), which indicates that the dry ageing time should be long enough to obtain enhanced sensory characteristics. Our results showed that some of the differences between ageing methods were only found after ageing for 19 days, e.g. water content of the inner layer, taste of salty and tenderness between samples using dry ageing and vacuum; in addition, the smell score of the raw meat after ageing differed between samples using the two dry ageing methods. Thus, it is important to be aware of the balance between quality improvement, e.g. flavour, and increased costs due to weight loss and a lower throughput when setting the ageing time in practice.

When combining the effects of ageing methods and ageing time in the multivariate analysis, three sensory dimensions could be interpreted from the PCA plot. The first sensory dimension characterized samples as being most savory, fatty-fried, juicy and easy to process orally. The second sensory dimension, opposite to the first dimension, was related to the more distinct boiled meat-metallic flavour and fibrous feel characters. In contrast, the third sensory dimension, orthogonal to dimensions one and two, could be defined as expressing samples with liver-like, animal-like and metallic odour notes. The samples aged in dry ageing methods for 19 days were shown to be most characteristic for dimension one, where the samples aged in dry ageing bags and vacuum for 8 days belonged to sensory dimension two. The samples aged

in vacuum for 19 days were most characteristic for the third sensory dimension.

6 Conclusions

DGAT1 was one of several suggested candidate genes for marbling that showed an effect in this group of young bulls in Sweden, whereas *LEP* and *SCD1* showed no association with variation in marbling but with meat colour traits. Moreover *CAPN1*, a candidate gene for meat tenderness, showed association with meat colour. The associations of polymorphisms in *CAPN1* and *CAST* genes with meat tenderness were confirmed in our study on young bulls of beef breeds in Sweden. Despite the fact that CAPN1:c.947 is a marker included in commercial DNA tests, the CAST:c.155 SNP was found to be the better marker for tenderness in our study. Taken together, our results confirm that the variation in these beef quality traits is clearly under genetic control. Although we observed only minor breed differences, the lack of consistent gene effects between studies indicates that association between markers and traits may not be consistent over breeds, possibly due to the somewhat different genomic background of breeds. This may have implications on the applicability across populations of SNP chips developed in only one or a few breeds.

The dry ageing bag, as an alternative to traditional unpackaged dry ageing, will make it possible to decrease meat ageing loss and microbial growth compared with traditional dry ageing. Meat aged in dry ageing bags has similar sensory characteristics as traditional dry ageing regarding most of the sensory attributes. Compared with vacuum ageing, the total product yield was lower after ageing meat in dry ageing bags. However, the dry ageing bags could produce meat with enhanced tenderness and juiciness, characteristics that are valued by consumers. By using dry ageing bags it is possible to produce dry-aged meat under more controlled conditions without negative effects on sensory or other quality attributes.

7 Future research

- Our study found associations of the tested SNPs with beef colour, although the present studies are still quite limited in size. More studies should be carried out to identify the causative mutations or to test the possible associations between polymorphisms and beef colour.

- With the access to chips with a high number of SNPs and with improved sequencing techniques, it would be of interest to do genome wide association analyses on the present data to get more and precise knowledge about the genetic effects on beef quality.

- In our studies, a large effect of ageing time was found. Future studies need to be carried out to determine the optimum ageing time when using dry ageing bags.

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