Colour Characteristics of Fresh Pork

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Abstract

The objective of the present thesis was to obtain more fundamental understanding of the mechanisms for variations in colour and colour stability of fresh pork. The effects of breed and genotype, including Duroc, Hampshire (three *RN* alleles), Landrace and Yorkshire pigs, were studied. Furthermore, effects of strategic feeding with creatine, pre-slaughter treatment, slaughter procedure and carcass cooling on muscle metabolism and ultimate colour and colour stability were studied.

It was concluded that the early progress in *post mortem* temperature and pH decline affect the colour of pork independent of whether this is triggered by variations in genotype, feed-induced changes, pre-slaughter stress or cooling rate. The level of oxymyoglobin was shown to be more important for the colour of pork during retail display, than the level of metmyoglobin. A high level of oxymyoglobin preserves high redness despite of oxidation to metmyoglobin. Ageing of pork for a week increased the ability of pork to stimulate oxygenation of deoxymyoglobin to oxymyoglobin, i.e. blooming, without compromising the colour stability during four days of subsequent retail display. Hence, ageing results in lighter, redder and more yellow pork colour.

It was further concluded that genetics giving rise to variation in the colour of pork is related to differences in pigment content and in muscle metabolism. The latter influences the redox status of the muscle and the activity of oxygen-consuming and metmyoglobin-reducing enzymes and accordingly the relative proportion of the myoglobin species. The colour of pork from Landrace pigs was generally lighter, redder and more yellow than pork from Duroc pigs due to higher level of oxymyoglobin at the meat surface, i.e. more intense blooming. The colour of pork from Hampshire pigs was highly related to the *RN* genotype. Pork from carriers of the RN^- allele was generally lighter, redder and more yellow than that from non-carriers of the RN^- allele, and the *rn** (V199I) allele tended to decrease redness and yellowness.

Keywords: colour characteristics, myoglobin species, blooming, pork, breed, *RN* genotype, pH decline, creatine, cooling, ageing

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Papers I-VI

The present thesis is based on the following papers, which will be referred to by their Roman numerals:

I. Lindahl, G., Lundström, K. and Tornberg, E. 2001. Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs. *Meat Science, 59*, 141-151

II. Lindahl, G., Enfält, A.-C., von Seth, G., Josell, Å., Hedebro-Velander, I., Andersen, H. J., Braunschweig, M., Andersson, L. & Lundström, K. 2004. A second mutant allele (V199I) at the *PRKAG3* (*RN*) locus-II. Effect on colour characteristics of pork loin. *Meat Science, 66*, 621-627

III. Lindahl, G., Karlsson, A.H., Lundström, K. & Andersen, H. J. 2005. Significance of storage time on degree of blooming and colour stability of pork loin from different crossbreeds. *Submitted for publication*.

IV. Lindahl, G., Henckel, P., Karlsson, A. H. & Andersen, H. J. 2005. Significance of early post mortem temperature and pH decline on colour characteristics of pork loin from different crossbreeds. *Submitted for publication*.

V. Lindahl, G., Young, J. F., Oksbjerg, N. & Andersen, H. J. 2005. Influence of dietary creatine monohydrate (CMH) and carcass cooling rate on colour characteristics of pork loin from different pure breeds. *Submitted for publication*.

VI. Lindahl, G., Enfält, A. C., Andersen, H. J. & Lundström, K. 2005. Impact of RN genotype and storage time on colour characteristics of the pork muscles *longissimus dorsi* and *semimembranosus* (Manuscript).

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Gunilla Lindahl's contribution to the papers

I. Planning the experiment, performing the colour measurements, evaluation of the results and writing the manuscript.
II. Planning the colour measurements, participation in the colour measurements, evaluation of the results and writing the manuscript.
III. Planning the experiment, performing the colour measurements, evaluation of the results and writing the manuscript.
IV. Evaluation of the results and writing the manuscript.
V. Planning the colour measurements, participation in the colour measurements, evaluation of the results and writing the manuscript.
VI. Planning the experiment, participation in the colour measurements, evaluation of the results and writing the manuscript

Appendix

List of abbreviations used in the text

AMP	adenosine monophosphate
AMPK	adenosine monophosphate-activated protein kinase
ATP	adenosine triphosphate
BF	*M. biceps femoris*
CIE	Commission Internationale de l'Eclairage
CMH	creatine monohydrate
CP	creatine phosphate
CO	carbon monoxide
D	Duroc breed
DFD	dark, firm and dry
EEL	reflectance value (Evans Electroselenium Ltd)
H	Hampshire breed
HAL	locus responsible for halothane sensitivity
H_2O_2	hydrogen peroxide
K/S	Kubelka-Munk scattering (S) and absorbance (K) coefficients
K/S value	calculated from Kubelka-Munk formula $K/S = (1-R)^2/2R$
L	Landrace breed
LD	*M. longissimus dorsi*
MAC	molar absorbance coefficient (ε)
Mb	deoxymyoglobin
MbO_2	oxymyoblobin
MetMb	metmyoglobin
NADH	reduced nicotineamide adenine dinucleotide
NN	genotype, devoid of the halothane sensitivity allele
Nn	genotype, heterozygous for the recessive halothane sensitivity allele
nn	genotype, homozygous for the recessive halothane sensitivity allele
NO	nitrogen oxide
O_2	oxygen
PCA	principal component analysis
PFN	pale, firm and non-exudative
PLS	partial least squares regression
PSE	pale, soft and exudative
PSS	porcine stress syndrome
R	reflectance
RFN	red, firm and non-exudative
RN	Rendement Napole
RN^-	199V-200Q allele in the *PRKAG3* gene on the *RN* locus, dominant
rn^+	199V-200R allele in the *PRKAG3* gene on the *RN* locus, wildtype
rn^*	199I-200R allele in the *PRKAG3* gene on the *RN* locus
RSE	red, soft and exudative
RYR1	mutation in the Halothane gene
SM	*M. semimembranosus*
Y	Yorkshire breed

Introduction

Meat pigments

Myoglobin and hemoglobin are the pigments giving meat the red colour (Govindarajan, 1973; Giddings, 1977). Myoglobin is distributed uniformly throughout the muscle, and its role is to store and to facilitate the diffusion of oxygen from the capillaries to the intracellular structures, where the oxygen is used for oxidative processes (Stryer, 1981; Ledward, 1992). Hemoglobin, which is contained in the red blood cells, serves as the oxygen carrier in blood (Stryer, 1981).

The myoglobin content in muscle depends on species, breed, sex, age, type of muscle, level of training (Ledward, 1992) and altitude (Gimenez *et al.*, 1977). Myoglobin is present in the sarcoplasmic fraction of the muscle (Govindarajan, 1973) and evenly distributed across the muscle fibres (Swatland, 1984). Consequently, the myoglobin content is higher in a red muscle, such as the inside part of porcine *M. biceps femoris* (BF), compared with white muscles, such as porcine *M. longissimus dorsi* (LD), and the outside part of porcine BF (Beecher *et al.*, 1965).

Myoglobin is a compact globular protein consisting of globin (apo protein) and an iron containing heme group, Fe-protoporphyrin, which is the chromophore of myoglobin (Stryer, 1981). The characteristic colours of myoglobin upon interaction with light depend on the ligand bound to the chromophore. Hemoglobin is composed of four globin molecules with four heme groups. The iron atom binds to the four nitrogens in the centre of the protoporphyrin ring and can form two additional bonds, one on either side of the heme plane, the fifth and sixth coordinating positions. The iron atom can be in several redox states with the ferrous (Fe^{2+}) and the ferric (Fe^{3+}) redox states being most important in relation to fresh meat colour. The fifth coordinating position is bound to histidine on the globin, and the sixth is free for binding to different small ligands such as O_2, H_2O, OH^-, NO and CO (Hamm, 1975; Stryer, 1981). The colour of myoglobin is determined by the redox state and by the type of ligand bound (Govindarajan, 1973; Hamm, 1975). Oxygen can only be bound to myoglobin in the ferrous redox state, whereas H_2O is bound in the ferric redox state at physiological pH and below (Govindarajan, 1973). The different myoglobin species in fresh meat are shown in Table 1.

Table 1. *Chemical species of myoglobin*

Myoglobin species	Oxidation state	Ligand	Colour
Deoxymyoglobin (Mb)	Fe^{2+}	None	Purple
Oxymyoglobin (MbO$_2$)	Fe^{2+}	O_2	Bright cherry red
Metmyoglobin (MetMb)	Fe^{3+}	H_2O [pH<8]	Brown

The heme group is a flat molecule bound to myoglobin in a hydrophobic crevice, protecting the iron from oxidation (Stryer, 1981). The iron is more prone to oxidation if the heme group is detached from the globin (Govindarajan, 1973).

Myoglobin has high affinity for oxygen and is saturated to 50% even at an oxygen partial pressure of 1 mm Hg and is nearly fully saturated at an oxygen partial pressure of 10 mm Hg (George & Stratmann, 1952).

The colour of fresh meat is determined by the concentration and chemical nature of the hemoproteins present (Govindarajan, 1973) and the muscle structure (Offer *et al*., 1989), which is influenced by temperature/pH history of the muscle post-slaughter (Ledward, 1992). In most meats, myoglobin is the main heme pigment although hemoglobin may be present in significant concentrations and to some extent mitochondrial cytochrome *c* (Hamm, 1975; Ledward, 1992). The colour of meat can be discussed focusing on myoglobin, as the different redox states of myoglobin and hemoglobin have nearly identical colour characteristics.

Myoglobin oxygenation, oxidation and reduction

The colour cycle of fresh meat is reversible and dynamic with constant interconversion of mainly the three species: deoxymyoglobin (Mb), oxymyoglobin (MbO_2) and metmyoglobin (MetMb) (Govindarajan, 1973). The surface colour changes from purple to bright red, due to oxygenation of Mb to MbO_2, when fresh meat is exposed to oxygen, a reaction known as blooming (Govindarajan, 1973; Giddings, 1977). The reaction is reversible with oxygen partial pressure determining the partition between the two species (Giddings, 1974). The oxygen binding of Mb only shows a small effect with changes in pH (Govindarajan, 1973). The ferrous species Mb and MbO_2 oxidise to ferric MetMb upon which the oxygenation ability is lost. MbO_2 is more stable to autoxidation (see below for explanation of the term) compared with Mb (Govindarajan, 1973; Stryer, 1981). The interconversion of the myoglobin species is shown in Fig. 1. The reduction of Mb is not reversible, but the dashed line indicates that it only becomes reversible in the prescence of a reductor, why MetMb is accumulated in the meat due to continous autoxidation in the inherent redox potential during storage. The development of MetMb at the meat surface depends essentially on the myoglobin autoxidation rate, enzymic MetMb reduction and oxygen consumption rate (Renerre, 1990).
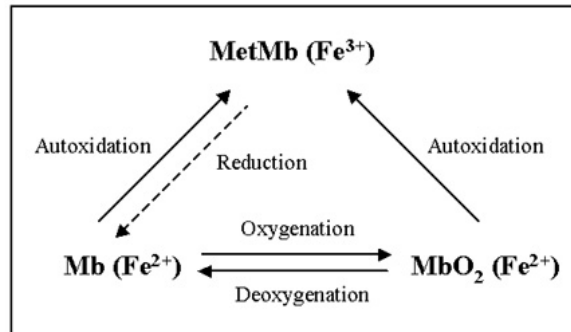
Fig 1. Interconversion of the myoglobin species Mb, MbO$_2$ and MetMb.

Oxygenation

When meat is freshly cut, the myoglobin is in the purple reduced form Mb. On exposure to air, myoglobin rapidly and reversibly combines with oxygen to form the bright cherry-red MbO$_2$ (Ledward, 1992). The myoglobin oxygenation is a first order reversible reaction (Bevilacqua & Zaritzky, 1986). The meat surface blooms to the bright cherry-red colour within minutes of exposure to air (Ledward, 1992) and with time, the small layer of MbO$_2$ spreads downwards into the meat (Feldhusen *et al.*, 1994b). The depth to which MbO$_2$ diffuses depends on the activity of the oxygen-consuming enzymes, i.e. the oxygen consumption rate of the meat, the temperature and external oxygen pressure (Ledward, 1992). MetMb-reducing activity may also be involved. The oxygen diffuses through the aqueous environment and enters the hydrophobic heme crevice to occupy the sixth co-ordination site. Blooming is more efficient under conditions that increase oxygen solubility and discourage enzymic activity, i.e. at low temperatures and low pH values. Thus meat that has been aged for several weeks in vacuum prior to exposure to air blooms more rapidly and intensely than fresh meat owing to some loss of activity of the oxygen-consuming enzymes (Ledward, 1992), and formation of a deeper MbO$_2$ layer (Feldhusen *et al.*, 1994b).

Oxygen consumption

Oxygen uptake in *post rigor* muscle results from tissue respiration, reaction with heme pigments and dissolution into tissue fluids (DeVore & Solberg, 1974). *Post mortem* muscle is not inert, and mitochondria continue to metabolise oxygen (Ashmore *et al.*, 1971; Cheah & Cheah, 1971; Ashmore, Parker & Doerr, 1972; Bendall, 1972; Bendall & Taylor, 1972; Atkinson & Follet, 1973; DeVore & Solberg, 1974; Lanari & Cassens, 1991; Madavi & Carpenter, 1993; Tang *et al*, 2005). Mitochondrial activity in *post mortem* muscle is enhanced by high pH values (Cheah & Cheah, 1971; Ashmore Parker & Doerr, 1972; Bendall & Taylor, 1972) and high storage temperature (Bendall, 1972; Bendall & Taylor, 1972), which simultaneously results in higher oxygen consumption. Moreover, the

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oxygen consumption rate is influenced by muscle type due to differences in muscle metabolism as shown in studies on beef (Bendall & Taylor, 1972; Lanari & Cassens, 1991; Madavi & Carpenter, 1993). However, as a consequence of respiration the oxygen consumption rate declines with time *post mortem* in porcine (Atkinson & Follet, 1973) and bovine muscle (Bendall & Taylor, 1972; Atkinson & Follet, 1973; Lanari & Cassens, 1991; Madavi & Carpenter, 1993; Tang *et al*., 2005). This decline is due to loss in structural integrity of the mitochondria (Cheah & Cheah, 1971; Tang *et al*, 2005), which subsequently allows oxygen to penetrate further into the muscle (Morley, 1971; Feldhusen *et al*., 1994b; Millar *et al*., 1994) and a more pronounced blooming (Feldhusen *et al*., 1994b; Zhu *et al*., 2001; Lindahl *et al*., 2005a, Paper VI; Lindahl *et al*., 2005c, Paper III) that gives rise to a more red colour (Rosenvold & Andersen, 2003a; Lindahl *et al*., 2005a,c, Paper VI, III).

Autoxidation

The oxidation of ferrous myoglobin (Fe^{2+}) to ferric myoglobin (Fe^{3+}) is termed autoxidation, since it proceeds with oxygen as the sole reducing species (Renerre, 1990). The rate of autoxidation increases with increasing temperature (Brown & Mebine, 1969), low pH (Gotoh & Shikama, 1974) and low oxygen pressure (George & Stratmann, 1952). The autoxidation rate has a maximum at an oxygen partial pressure of 1 mm Hg, and the rate decreases when the oxygen partial pressure increases up to 30 mm Hg (George & Stratmann, 1952). Above 30 mm Hg the oxygen pressure has little or no effect on the rate of autoxidation (George & Stratmann, 1952).

Wazawa *et al*. (1992) and Shikama (1998) proposed possible pathways for the complex mechanism of myoglobin oxidation by molecular oxygen. H_2O_2 plays a key role in the complicated reactions (Wazawa *et al*., 1992). H_2O_2 is formed in the normal autoxidation of MbO_2 to MetMb through dismutation of superoxide anion (O_2^-) generated in the reaction. Most of the formed H_2O_2 is eliminated by MetMb through a cyclic formation of myoglobin species with iron in the somehow unusual +4 redox state, ferryl species. Under air-saturated conditions almost all myoglobin exists in the oxy-form, MbO_2, and most of the H_2O_2 formed is eliminated. However, the deoxy-form Mb is the most preferred target for H_2O_2, and the amount of Mb, that is in equilibrium with MbO_2, is an important factor for the overall stoichiometry of the myoglobin autoxidation. The amount of Mb increases rapidly with decreasing partial pressure of O_2, and H_2O_2 then preferably reacts with Mb. H_2O_2 oxidises two equivalents of Mb to MetMb, which explains why the rate of formation of MetMb increases with decreasing partial pressure of O_2.

In pure solution the autoxidation of MbO_2 is first order with respect to unoxidised myoglobin (George & Stratmann, 1952; Brown & Mebine, 1969; Shikama & Sugarwara, 1978; Renerre, Anton & Gatellier, 1992) and depends on several external parameters including pH (Shikama & Sugawara, 1978), oxygen pressure (George & Stratmann, 1952), ionic strength in acidic solution (pH<6.5) (Andersen, Bertelsen & Skibsted, 1988) and light (Bertelsen & Skibsted, 1987). However, results found for myoglobins in solution cannot be applied

indiscriminately to meat as in meat (1) a reducing system capable of reducing MetMb to the ferrous state is present, (2) a catalytic process operates and (3) different gradients of oxygen concentration (from 0 to external pressure) will be present in various muscles (Ledward, 1992). Furthermore, biological factors such as muscle type (Renerre, 1990) may also play an important role in the autoxidation rate.

A few millimeters below the meat surface, a brown layer develops due to the accelerated formation of MetMb (Govindarajan, 1973; Giddings, 1977), as the partial pressure of oxygen in this region is in the optimum range for formation of MetMb. The main discoloration process proceeds from this MetMb layer. Under the MetMb layer is the purple colour characteristic of Mb (Govindarajan, 1973).

MetMb reduction

It is now accepted that the reduction processes in meat are primarily enzymatic in nature with NADH as coenzyme. The pool from where NADH comes is not yet known and may involve mitochondria and/or submitochondrial particles (Renerre, 1990). The reduction of MetMb in meat can take place under both anaerobic and aerobic conditions (Ledward, 1992). MetMb reductase activity has been shown in porcine LD (Mikkelsen *et al.*, 1999). The enzymatic reduction was NADH-dependent, and MetMb was also reduced by NADH in a non-enzymatic reaction. The rate of enzymatic reduction of MetMb in porcine muscle depends strongly on pH and ionic strength (Mikkelsen & Skibsted, 1992).

MetMb-reducing activity has been shown in sarcoplasmic fraction of porcine muscle (Mikkelsen, Juncher & Skibsted, 1999), sarcoplasmic (Renerre & Labas, 1987; Echevarne, Renerre & Labas, 1990; Reddy & Carpenter, 1991) and microsomal fractions of bovine muscles (Echevarne *et al.*, 1990) and in sarcoplasmic and particulate fractions (including myofibrils, mitochondria and other parts of the muscle cell) of ovine muscles (Bekhit, *et al.*, 2001; Bekhit *et al.*, 2003). Bovine MetMb-reducing acitvity is influenced by muscle type (Renerre & Labas, 1987; Reddy & Carpener, 1991; Madavi & Carpenter, 1993).

Arihara *et al.* (1995) localised a MetMb-reducing enzyme system in bovine skeletal muscle and proposed a mechanism for MetMb reduction. The components NADH-cytochrome b_5 reductase, cytochrome b_5 and outer membrane cytochrome b are localised in the mitochondrial and microsomal fractions. NADH-cytochrome b_5 reductase reduces MetMb by using outer mitochondrial membrane cytochrome b at the mitochondrial surface and, in part, by using cytochrome b_5 at the sarcoplasmic reticulum. Moreover, Lynch *et al.* (1998) have suggested a potential reaction whereby α-tocopherol maintains MbO_2 via enhancement of cytochrome b_5-mediated reduction of MetMb. However, they concluded that the observed *in vitro* reduction was relatively small, which may support only a minor role for this colour-stabilising mechanism mediated by α-tocopherol in meat. The extent, to which this may actually occur in meat, requires further study.

Loss of reducing activity in meat during storage is due to the combination of factors including fall in tissue pH, depletion of required substrates and co-factors, pH-induced denaturation of the enzymes and ultimately complete loss of structural

integrity and functional properties of the mitochondria (Giddings, 1974). Decrease in MetMb reductase activity with time *post mortem* has been observed in porcine (Zhu & Brewer, 1998a) and bovine muscles (Madavi & Carpenter, 1993). In contrast, (Bekhit *et al*, 1991) reported increase in MetMb reductase activity upon storage of ovine muscles.

Reflectance and meat structure

When light reaches the surface of pork, some of it is reflected from the wet surface with no change in wavelength, i.e. specular reflectance (Swatland, 1992). In contrast, most of the light that enters the meat is strongly scattered, absorbed (much of the green light is absorbed by myoglobin) and returned so that the pork appears pink to the observer (Offer *et al*., 1989; Swatland, 1992). If a high degree of scattering shortens the light path through the meat, the opportunity for the selective absorbance by myoglobin is decreased, and the meat is less pink than normal, as well as more pale than normal due to scattering (Offer *et al*., 1989; Swatland, 1992). Consequently, the surface reflectance spectrum of meat is a result of the selective absorbance mainly by myoglobin imposed upon a spectrum formed by the scattered light that escapes from meat (Swatland, 1994). In combination, these two factors may cause the almost white appearance of severe PSE pork. Moreover, the increased light scattering in severe PSE pork originates from denaturation of sarcoplasmic proteins (Swatland, 1992).

However, protein denaturation is not responsible for the well-known relationship between paleness and pH that is observed in all types of fresh meat across a wide pH range, from pH 7 to pH 5 (Swatland, 1992). This may be due to the fact that increased separation of myofibrils increases the chances of scattering at the surface of myofibrilles (Offer *et al*., 1989) and to the birefringence of myofibrils (Offer *et al*., 1989; Swatland, 1992, 1995, 2002). When light passes through myofibrils, it takes two routes of different lengths, and the path difference of the two rays is strongly affected by pH. Thus, changes in the refractive index within the fibril might also be a cause of the pH-related increases in light scattering (Swatland, 1992).

The monochromatic fibre optic probe (FOP), which measures the internal reflectance of meat, has a peak sensitivity at 900 nm, where the absorbance by red heme pigments is minimal (Bendall & Swatland, 1988). The FOP value increases slowly as pH falls from 7 to 6.3 and increases more rapidly as pH falls to about 5.4, where it reaches a maximum value. The most likely explanation is that the increase in FOP value is related to increased space between myofibrils due to the decrease in myofilament spacing as negative-charge repulsion decreases towards the isoelectric point at about pH 5.4. The myosin filaments of pork LD are separated by a distance of about 46 nm early *post mortem*, and the distance decreases to 41 to 42 nm when pH of the muscle has fallen to 5.2-5.5 3 to 6 hours *post mortem* (Bendall & Swatland, 1988).

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Colour perception

Colour (in the psychophysical sense) is that characteristic of a visible radiant power (light) by which an observer may distinguish differences between two structure-free fields of view of the same size and shape, such as may be caused by differences in the spectral composition of the radiant power (colour stimulus) entering the eye (Wyszecki & Stiles, 1982).

- Colour stimulus is defined as radiant power (light) of a given magnitude and spectral composition, entering the eye and producing a sensation of colour (Wyszecki & Stiles, 1982).

- Monochromatic stimulus is defined as monochromatic radiant power (light) of a given magnitude and wavelength (or frequency) entering the eye and producing a sensation of light or colour (Wyszecki & Stiles, 1982).

- Achromatic stimulus is defined as the colour stimulus chosen because it usually yields a colour perception that is devoid of hue under the desired observing conditions (Wyszecki & Stiles, 1982).

Colorimetry

Colorimetry is the branch of colour science concerned with specifying numerically the colour of a physically defined visual stimulus in such a manner that (1) when viewed by an observer with normal colour vision, under the same observing conditions, stimuli with the same specification look alike, (i.e. are in complete colour-match), (2) stimuli that look alike have the same specification, and (3) the numbers comprising the specifications are continuous functions of the physical parameters defining the spectral radiant power distribution of the stimulus (Wyszecki & Stiles, 1982).

The experimental laws of colour matching are summed up in the empirical *trichromatic generalization* (Wyszecki & Stiles, 1982). This states that over a wide range of conditions of observation, many colour stimuli can be matched completely in colour by additive mixtures of three fixed primary stimuli whose radiant powers (light) have been suitably adjusted. Other colour stimuli have to be mixed with one of the primary stimuli before a complete colour match with a mixture of the other two primary stimuli can be obtained. Additive mixtures mean a colour stimulus for which the radiant power in any wavelength interval, small or large, in any part of the spectrum is equal to the sum of the powers in the same interval of the constituents of the mixture, constituents that are assumed to be optically incoherent.

Observational conditions

In colour matching, the two stimuli are normally presented as contiguous light patches of similar shape and area (Wyszecki & Stiles, 1982). However, a colour match of two stimuli with different spectral radiant power distributions, valid for a given observer when looking directly at the centre of the matching field, will not generally remain valid if the observer looks to the side. Changing the area of

matching may also upset the match. These viewing conditions must be fixed if critical colour measurements are to be made and thus Standard Colorimetric Observer, Standard Illuminants and Standard Illuminating and Viewing Conditions were defined by the Commission Internationale de l'Eclairage (CIE).

CIE Standard Observer

In 1931 CIE specified the first *CIE Standard Colorimetric Observer* (2° angular viewing), where the colour matching properties are defined by the colour matching functions $\overline{x}(\lambda)$, $\overline{y}(\lambda)$, $\overline{z}(\lambda)$ (Wyszecki & Stiles, 1982). In 1964, CIE recommended an alternative set of standard colour-matching functions denoted $\overline{x}_{10}(\lambda)$, $\overline{y}_{10}(\lambda)$, $\overline{z}_{10}(\lambda)$ with larger angular viewing (10°) referred to as the *CIE 1964 Supplementary Standard Colorimetric Observer*. The 10° standard observer now dominates.

CIE Standard Illuminants

CIE recommended a set of spectral radiant power distributions (illuminants) called CIE standard illuminants, and several standard illuminates exist (Wyszecki & Stiles, 1982). CIE standard illuminant A represents light from the full radiator at absolute temperature 2856 K. CIE standard illuminant D_{65} represents a phase of natural daylight with a correlated colour temperature of approximately 6504 K. The CIE standard illuminant B was intended to represent direct sunlight with a correlated colour temperature of approximately 4874 K, and CIE standard illuminant C was intended to represent average daylight with a correlated temperature of approximately 6774 K. Both illuminant B and C are, however, considered inadequate in representing the intended phases of natural daylight and are consequently not used today. CIE recommended standard illuminant D_{65} in 1964, and it is now widely used as the representative of average daylight for colorimetry.

Standard Illuminating and Viewing Conditions

The CIE recommends four different illuminating and viewing conditions for reflectance measurements (Wyszecki & Stiles, 1982): (1) 45/0-condition: Illumination at an angle of 45 ± 5° from the normal of the sample surface. Viewing at an angle not exceeding 10° from the normal to the sample; (2) 0/45-condition: Illumination at an angle not exceeding 10° from the normal of the sample surface. Viewing at an angle of 45 ± 5° from the normal to the sample; (3) d/0-condition: The sample is illuminated diffusively by an integrating sphere. The angle between the normal to the sample and the axis of the viewing bean should not exceed 10°; (4) 0/d-condition: The sample is illuminated by a beam whose axis is at an angle not exceeding 10° from the normal to the sample. The reflected light is collected by an integrating sphere.

Colour scales

The CIE colorimetric system includes computational methods designed to aid in the prediction of the magnitude of the perceived colour difference between two objects (Wyszecki & Stiles, 1982). The colour is described in a colour space, and it is important that the colour space is uniform over the whole range. CIE recommends the approximately uniform CIE 1976 (*L*a*b*)-space. The Hunter 1958 (*Lab*)-space is another uniform colour space. These two colour scales are widely used in meat science, but it is important to notice that the *L*, *a*, *b* and the *L*, *a*, *b* values are not identical.

Both the (*L*a*b*)-space and (*Lab*)-space are based on the theory of counter-colours. The principle of the *L*, *a*, *b* colour scale is described in Fig 2. The *L* value represents lightness where $L^* = 0$ is completely black, and $L^* = 100$ is completely white. The *a* value represents red-green colours: positive *a* values mean red colours and negative *a* values mean green colours. The *b* value represents yellow-blue colours: positive *b* values mean yellow colours and negative *b* values mean blue colours.
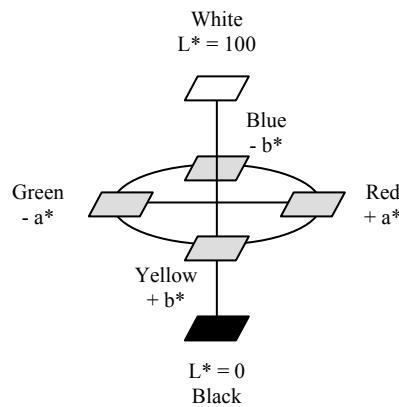

Fig. 2. Principle of the CIE 1976 *L*a*b* colour space.

Chroma is the attribute of visual sensation, which permits a judgement to be made of the degree to which a chromatic stimulus differs from an achromatic stimulus of the same brightness (Wyszecki & Stiles, 1982). Chroma (*C*) is numerically quantified as $[(a^*)^2 + (b^*)^2]^{1/2}$ in the CIE 1976 (*L*a*b*) colour space.

Hue is the attribute of a colour perception denoted by blue, green, yellow, red, purple and so on (Wyszecki & Stiles, 1982). Hue is numerically quantified by the hue angle (*h*) defined as arctan *b*/*a* in the CIE 1976 (*L*a*b*) colour space.

Meat colour measurements

The colour of meat has been assessed by measuring tristimulus parameters ($L*$, $a*$, $b*$ values) (Honikel, 1997, 1998), by image analysis (O'Sullivan et al., 2003) and by methods using reflectance spectra (Snyder, 1965; Stewart, Zipzer & Watts, 1965; Franke & Solberg, 1971; van den Oord & Wesdrop, 1971; Eagerman, Clydesdale & Francis, 1978; Krzywicki, 1979; Bevilacqua & Zaritzky, 1986; Mancini, Hunt & Kropf, 2003) and finally by use of absorbance spectra of extracted myoglobin (Krzywicki, 1982; Tang, Faustman & Hoagland, 2004).

Tristimulus parameters

The $L*$, $a*$, $b*$ values describe the colour of meat, but they do not reveal the origin of the observed colour in terms of myoglobin species. The colour parameters vary according to the myoglobin species (Fernández-López et al., 2000; Lindahl et al., 2001, paper I), and a change in $a*$ or $b*$ value may be an effect of oxygenation-deoxygenation or an effect of oxidation to MetMb (Johansson, 1989). Thus there is a need for assessing the myoglobin species as a complement of $L*$, $a*$, and $b*$ values. Bloom time affects the colour characteristics (Brewer et al., 2001; Zhu, Bidner & Brewer, 2001; Lindahl et al., 2005c, Paper III), and it is important not to measure meat colour until blooming is complete. At least 1 hour at 4°C (Honikel, 1997) or preferably 2 hours at 3°C (Honikel, 1998) are recommended in reference methods for assessment of meat colour.

Image analysis

O'Sullivan et al. (2003) used a digital camera to estimate meat colour. Red (R), green (G) and blue (B) values were measured from digital photographic images. An algorithm was used to convert a given area of the picture to mean RGB and $L*$, $a*$, $b*$ values. The measurements by the digital camera were highly correlated to sensory colour analysis. This was postulated to be due to the fact that the camera took measurements over the entire surface of the sample and thus was more representative than meaurements by a colorimeter.

Assessment of myoglobin species

The assessment of myoglobin species using reflectance or absorbance spectra is based on the differing spectra of the myoglobin species Mb, MbO_2 and MetMb (Fig. 3). Isobestic points, where two or all three of the myoglobin species have the same reflectance/absorbance, are used (Krzywicki, 1979, 1982; Hunt et al., 1991; Mancini, Hunt & Kropf, 2003; Tang, Faustman & Hoagland, 2004). Mb may be determined at 473 nm, isobestic point of MbO_2 and MetMb; MbO_2 at 610 nm, isobestic point of Mb and MetMb; MetMb at 572 nm, isobestic point of MbO_2 and Mb. The isobestic point of all the three myoglobin species, 525 nm, is often used to eliminate differences in myoglobin content between different meat samples.
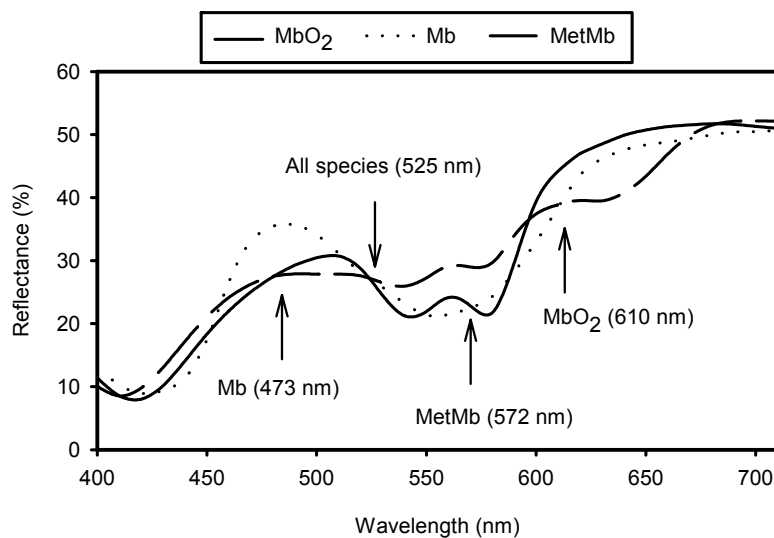
Fig 3. Reflectance spectra of Mb, MbO$_2$ and MetMb

Quantifying myoglobin species with absorbance measurements of extracted myoglobin has some disadvantages compared with reflectance measurements on the meat surface. The results depend on how deep the samples are taken, as the layers of the different myoglobin species may vary, and on possible changes (oxidation) during extraction. Another drawback is that the physical characteristics of the meat are not taken into account.

Methods using Kubelka-Munk formula

The reflectance values have often been converted to Kubelka-Munk K/S values, which makes the data more linear and helps accounting for the scattering (S = scattering coefficient) and absorptive (K = absorbance coefficient) properties of the meat (Francis & Clydesdale, 1975 cited by Hunt *et al.*, 1991). The K/S value is calculated from the Kubelka-Munk formula $K/S=(1-R)^2/2R$. The R value is defined as the reflectance of a meat sample so thick that further increases in thickness fail to change the reflectance. A linear relationship has been shown between K/S 525 and pigment concentration. Often, the ratio K/S 473 to K/S 525 has been used for estimation of percentage of Mb and the ratio K/S 572 to K/S 525 for percentage of MetMb, whereas the percentage of MbO$_2$ has been estimated by the difference from 100% (Hunt *et al.*, 1991). An alternative for determining the percentage of MbO$_2$ directly by using the ratio K/S 610 to K/S 525 was recommended by Mancini, Hunt & Kropf (2003).

The methods using K/S-ratios at different wavelengths require reflectance measurements on meat with 100% Mb, MbO$_2$ and MetMb, respectively, to calculate percentage of the myoglobin species (Hunt *et al.*, 1991). However, it is not always possible to fully convert the meat to 100% of these myoglobin species, especially not meat with a high pH. Moreover, these measurements have to be

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made on three different meat slices, which may differ. The method of Krzywicki (1979; se below) is more straightforward as the calculations proceed from the reflectance measurements of the actual meat and are not affected by the pH of the meat.

Krzywicki method

The method of Krzywicki (1979) to assess the relative fractions of the myoglobin species Mb, MbO_2 and MetMb is based on measurements of reflex attenuance (log 1/R) of incident light at the isobestic points 572, 525, 473 and 730 nm. The latter value corresponds to the achromatic attenuance of light at the meat surface and is used as an objective measure of its lightness. The term reflex attenuance is used to distinguish between attenuation of light due to both absorption and diffusion and that caused by light absorption by transparent materials (absorbance) only. The reflex attenuance of light falling on a meat surface is the sum of two terms: (1) achromatic absorption caused by refraction and internal reflection at the structural elements of the meat and (2) fraction of light absorbed by pigments present in the tissue. The achromatic absorption of light depends on the light-diffusing properties of muscle proteins, cell membranes and fat particles. It increases in media of low-diffusing power, with an increase of light path length, as well as in well-hydrated meat of high pH, and decreases with growth of visible marbling or protein denaturation. Absorption maxima of Mb, MbO_2 and MetMb lie in the band 400 to 630 nm, with a minimum at approximately 730 nm, where it is independent of the pigment concentration and can be taken as the attenuation of light by pigment-free meat. The fractions of Mb and MetMb are calculated from reflectance values using the Lambert-Beer law and molar absorbance coefficients for the different myoglobin species. The fraction of MbO_2 is calculated by difference from 1.

Colour of pork

The colour of fresh pork is influenced by intrinsic factors such as breed, genotype, gender, type of muscle, dietary supplementation with vitamin E or creatine, and extrinsic factors such as pre-slaughter handling and slaughter procedure, which influence pH decline and ultimate pH, and furthermore, storage time and conditions (temperature, atmosphere, light etc.) (Faustmann & Cassens, 1990; Renerre, 1990; Sellier & Monin, 1994; Rosenvold & Andersen, 2003b).

Genetic background

Genetics and pre-slaughter handling are important factors for the variation in technological and sensory quality of pork (Sellier & Monin, 1994). Two types of genes, polygenes and major genes, are distinguished among genes affecting meat quality traits. Polygenes are defined as genes whose individual effect on a trait is small relative to the total variance of this trait. A gene is considered a major gene when the difference between the mean values of the individual homozygotes for this gene is equal or superior to one phenotypic standard deviation of the trait. Two major genes affecting meat quality traits have been identified in the pig: the Halothane gene and the *RN* gene (Sellier & Monin, 1994). The abbreviation *RN*

stands for Rendement Napole (Napole yield; Naveau, Pommert & Lechaux., 1985).

Halothane gene

Briskey (1964) indicated that pale, soft and exudative (PSE) proneness had a marked genetic component, since certain pig breeds, or certain strains within these breeds, contained a large proportion of PSE-prone animals, whereas other breeds or strains were practically devoid of the defect (Ludvigsen, 1954). PSE proneness was shown to be closely associated to porcine stress syndrome (PSS), as reviewed by Briskey (1954), and PSS can be triggered by stress (O'Brien, 1987). Christian (1974) and Eikelenboom & Minkema (1974) found that PSS could be triggered by the anaesthic gas halothane, which has been used for phenotyping with regard to PSS sensitivity. The locus responsible for halothane sensitivity was called HAL (Andresen & Jensen, 1977) with two alleles *N* (normal, dominant) and *n* (halothane sensitivity, recessive) (Minkema, Eikelenboom & van Eldik, 1977). However, it is questioned if the *n* allele is fully recessive (Sellier & Monin, 1994). The causative mutation (RYR1) for the Halothane gene is in the gene encoding for a ryanodine receptor isoform (Fujii *et al*., 1991), and the defect is associated to the calcium release in muscle (Endo *et al*., 1983; Ohnishi, Taylor & Gronert, 1983; O'Brien, 1986; Fill *et al*., 1990).

It is widely accepted that halothane sensitivity induces an acceleration of the *post mortem* pH decline, and consequently increases the frequency of PSE meat (Sellier & Monin, 1994). The colour of pork is strongly influenced by the *post mortem* pH decline and ultimate pH (Warris & Brown, 1987; Bendall & Swatland, 1988), which is shown also in meat of normal quality (Lindahl *et al*., 2001, Paper I; Støier *et al*., 2001; Rosenvold & Andersen, 2003a; Lindahl *et al*. 2004b, Paper II; Lindahl *et al*., 2005a,b,d, Paper VI, IV, V). The reported effect of the halothane gene on pork quality is varying, which seems to be related to whether the heterozygotes are fully recessive or not (Sellier & Monin, 1994). The effect of genotype on pork colour is reported to depend on several factors, such as pre-slaughter treatment (Klont & Lambooy, 1995a; Channon, Payne & Warner, 2000), stunning method (Channon, Payne & Warner, 2000) and on the muscle studied (Tam *et al*., 1998; Fischer, Mellet & Hoffman, 2000; Ohene-Adjei *et al*, 2003).

Moelich *et al*. (2003) reported no difference in colour of pork loin between the halothane genoypes. However, generally homozygotes for the normal *N* allele of the Duroc, Hampshire, Landrace and Yorkshire pure breeds or crossbreeds show darker muscle colour compared with heterozygotes or homozygotes for the recessive *n* allele in LD (Lundström *et al*., 1989; Sather *et al*., 1991; Klont *et al*., 1993; Klont & Lamboy, 1995a; Tam *et al*., 1998; Jeremiah *et al*., 1999; Channon, Payne & Warner, 2000, Hamilton *et al*., 2000; Apple *et al*., 2002; Ohene-Adjei *et al*, 2003) and in SM (Fischer, Mellet & Hoffman, 2000), although Tam *et al*. (1998) found no diffference in lightness between the *NN* and *Nn* genotypes in LD or between the *NN* and *nn* genotypes in SM.

Most studies do not report difference in redness between the genotypes in LD (Klont *et al*., 1993; Tam *et al*., 1998; Hamilton *et al*., 2000; Ohene-Adjei *et al*, 2003) or in SM (Tam *et al*., 1998; Fischer, Mellet & Hoffman, 2000). However,

Apple *et al.* (2002) found higher redness in LD of the *NN* genotype compared with LD of the *Nn* genotype, whereas Klont & Lamboy (1995a) reported lower redness in LD of the *NN* genotype compared with LD of the *nn* genotype. Several studies report no difference in yellowness in LD (Klont & Lamboy, 1995a) or in SM (Tam *et al.*, 1998; Fischer *et al.*, 2000; Ohene-Adjei *et al*, 2003), whereas other studies show lower yellowness in LD of the *NN* genotype compared with LD of the *Nn* genotype (Hamilton *et al.*, 2000; Apple *et al.*, 2002; Ohene-Adjei *et al*, 2003) or compared with LD of the *nn* genotype (Ohene-Adjei *et al*, 2003).

Tam *et al.*, (1998) found lower pigment content in the *NN* genotype compared with the *Nn* genotype in the dark part of SM, but no differences in pigment content between the halothane genotypes in the light part of SM or in LD, in line with Klont & Lamboy (1995a). Klont *et al.* (1993) reported a tendency to highest pigment content in LD of the *NN* genotype, lower in *Nn* muscle and lowest in *nn* muscle.

RN gene

The presence of a dominant mutation (denoted RN^-) with large effects on meat quality and processing yield in Hampshire pigs was first recognised by segregation analysis of phenotypic data by Naveau (1986) and Le Roy *et al.* (1990). A major locus (*RN*) affecting muscle glycogen content has been located to pig chromosome 15 (Milan *et al.*, 1995; Looft *et al.*, 1996; Mariani *et al.*, 1996). Milan *et al.* (2000) showed that the RN^- mutation is a nonconservative substitution (R200Q) at the *PRKAG3* locus on chromosome 15, which encodes a muscle-specific isoform of the regulatory γ subunit of adenosine monophosphate-activated protein kinase (AMPK). AMPK has a key role in regulating energy metabolism in eukaryotic cells and is activated by an increase in the ratio of adenosin monophosphate (AMP) to adenosin triphosphate (ATP). Activated AMPK is expected to inhibit glycogen synthesis and stimulate glycogen degradation. Milan *et al.* (2000) suggest that R200Q may be an activating mutation and a dominant negative mutation inhibiting glycogen degradation. The RN^- mutation has only been found in Hampshire pigs or crossbred pigs including Hampshire, and not in any other pig breed (Milan *et al.*, 2000).

A third allele (V199I) at the *PRKAG3* (*RN*) locus has been identified in the pig breeds Hampshire, Large White and Wild Boar (Milan *et al.*, 2000). Ciobanu *et al.* (2001) found the new allele in the pig breeds Landrace, Large White, Berkshire, Duroc and Duroc Synthetic and reported that it had effects on glycogen content and certain meat quality traits, such as ultimate pH and colour.

Three functionally significant alleles have been identified at the *PRKAG3* (*RN*) locus. They are as follows: 199V-200R (wildtype, *rn*⁺), 199V-200Q (RN^-) and 199I-200R (denoted *rn** in this thesis). The allele 199I-200R is associated with low level of glycogen, lactate and glycolytic potential and with high muscle pH, whereas the 199V-200R allele is associated with higher glycogen content and lower muscle pH (Milan *et al.*, 2000; Ciobanu *et al.*, 2001). Furthermore, the 199V-200Q allele is associated with the highest glycogen content and lowest pH.

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Most research carried out on the effects of the RN^- allele has been done on animals either classified as carriers or non-carriers of the RN^- allele based on phenotypic classification (Lundström & Andersson, 2001). However, in French studies, pigs being homozygous or heterozygous for the RN^- allele were differentiated using knowledge of the genotype of the sire and dam (Lebret *et al*., 1999; Le Roy *et al*., 2000). With the introduction of a DNA test (Milan *et al*., 2000; Ciobanu *et al*., 2001), it is now possible to determine genotypes of the animals. Sensory quality of pork loin including the three identified RN alleles has been studied by Josell *et al*. (2003a), technological meat quality by Lindahl *et al*. (2004a) and colour characteristics by Lindahl *et al*. (2004b, Paper II) and Lindahl *et al*. (2005a, Paper VI).

Breeds and gender

Muscle from the Hampshire breed is known to have a higher glycogen content than muscle from other breeds, especially in glycolytic muscles as reviewed by Lundström & Andersson (2001), and a low ultimate pH (Monin & Sellier, 1985; Sellier & Monin, 1994; Suuronen, 1995; Lindahl, *et al*., 2001, Paper I). These traits in the Hampshire breed are related to the presence of the dominant RN^- allele (Le Roy *et al*., 1990; Lebret *et al*., 1999; Le Roy *et al*., 2000; Lindahl *et al*., 2004a). The rate of the *post mortem* pH fall was earlier considered to be normal (Monin & Sellier, 1985; Sellier & Monin, 1994), while more recent studies have shown a slightly faster pH fall related to carriers of the RN^- allele (Josell, Martinsson & Tornberg, 2003b; Josell, von Seth & Tornberg, 2003c; Lindahl *et al*., 2004a; Lindahl *et al*., 2005a, Paper VI). The high glycogen content in carriers of the RN^- allele affects chemical composition and technological meat quality. RN^- carriers have lower protein content (Monin *et al*., 1992; Estrade, Vignon et Monin, 1993; Enfält *et al*., 1997a; Lindahl *et al*., 2004a), ultimate pH (Lundström, Andersson & Hansson, 1996; Enfält *et al*., 1997a; Lebret *et al*., 1999; Miller *et al*., 2000; Lindahl *et al*., 2004a), water-holding capacity and processing yield (Lundström, Andersson & Hansson, 1996; Enfält *et al*., 1997a; Lundström *et al*., 1998; Miller *et al*., 2000; Lindahl *et al*., 2004a) and higher reflectance values (Lundström, Andersson & Hansson, 1996; Enfält *et al*., 1997a; Miller *et al*., 2000; Lindahl *et al*., 2004b, Paper II). The RN^- carriers also have a higher degree of protein denaturation compared with non-carriers (Lundström, Andersson & Hansson, 1996).

Variations in meat colour between pig breeds have been observed (Lundström, 1975; Fjelkner-Modig & Persson, 1986a; Fjelkner-Modig & Tornberg, 1986b; Johansson, Andersson & Lundeheim, 1987; Langlois & Minivielle, 1989; Cameron, *et al*., 1990; Hammell, Laforest & Dufor, 1994; Oliver *et al*., 1994; Suuronen, 1995; Candek-Potokar, Zlender & Bonneau, 1998; Blanchard *et al*., 1999; Newcom *et al*., 2004; Lindahl *et al*., 2001, Paper I; Lindahl *et al*., 2005b,c,d, Papers IV, III, V). Pork from purebreed or crossbreed Duroc pigs was generally darker than pork from Landrace or Yorkshire pigs (Langlois & Minivielle, 1989; Cameron, *et al*., 1990; Oliver *et al*., 1994; Candek-Potokar, Zlender & Bonneau, 1998; Blanchard *et al*., 1999; Newcom *et al*., 2004; Lindahl *et al*., 2005c,d, Papers III, V), which was related to higher ultimate pH in Duroc pork in some of the studies (Candek-Potokar, Zlender & Bonneau, 1998; Newcom

et al., 2004; Lindahl *et al*., 2005d, Paper V), whereas no difference in ultimate pH was reported in others (Langlois & Minivielle, 1989; Cameron, *et al*., 1990; Oliver *et al*., 1994; Blanchard *et al*., 1999; Lindahl *et al*., 2005c, Paper III). Several studies showed more red colour in Duroc pork compared with pork from Landrace or Yorkshire pigs (Cameron, *et al*., 1990; Candek-Potokar, Zlender & Bonneau, 1998; Newcom *et al*., 2004), whereas other studies report less red colour (Oliver *et al*., 1994; Lindahl *et al*., 2005d, Paper V) or no difference in redness (Hammel, Laforest & Dufor, 1994; Lindahl *et al*., 2005c, Paper III).

Hampshire loin is found to be redder than Landrace (Hammel, Laforest & Dufor, 1994; Suuronen, 1995; Lindahl *et al*., 2001, Paper I) and Yorkshire loin (Lindahl *et al*., 2001, Paper I). Furthermore, Hampshire loin is reported as lighter (Hammel *et al*., 1994; Lindahl *et al*., 2001, Paper I) or darker (Fjelkner-Modig, 1986a; Suuronen, 1995) or not different in lightness (Lindahl *et al*., 2001, Paper I) compared with Landrace loin. Langlois & Minivielle (1989) reported no difference in lightness between Hampshire, Duroc and Yorkshire loin, but all were darker than Landrace loin.

Variation in meat colour within the Hampshire breed is attributed to differences between carriers and non-carriers of the RN^- allele (Lundström, Andersson & Hansson, 1996; Enfält *et al*., 1997a, b; Lundström *et al*., 1998; Lebret *et al*., 1999; Hamilton *et al*., 2000; Le Roy *et al*., 2000; Moeller *et al*., 2003; Lindahl *et al*., 2004b, Paper II; Lindahl *et al*., 2005a, Paper VI), although Miller *et al*. (2000) and Brewer *et al*. (2002) reported no effect of the RN^- allele on pork colour. The new allele V199I has also been reported to influence the colour of pork from the Hampshire (Fields *et al*., 2002; Plastow *et al*., 2002; Lindahl *et al*., 2004b, Paper II) and Landrace breeds (Ciobanu *et al*., 2001).

No difference in EEL reflectance value (Evans Electroselenium Ltd) was found between *i*) entire male, castrated male and female Swedish Landrace pigs (Malmfors & Nilsson, 1978), *ii*) castrated male and female Swedish Landrace and Yorkshire pigs (Lundström, 1975) or *iii*) entire male and female Yorkshire pigs (Lundström *et al*., 1987), whereas Enfält *et al*. (1997b) reported slightly higher EEL values in entire male compared with female Hampshire pigs. Castrates had slightly darker meat colour than entire males and females of Duroc, Hampshire, Landrace and Yorkshire breeds (Jeremiah *et al*., 1999), whereas gilts had darker meat colour than castrates of Duroc, Hampshire, Landrace and Yorkshire crossbreeds (Langlois & Minivielle, 1989). Eikelenboom & Hoving-Bolink (1993) and Hammel, Laforest & Dufor (1994) found no difference in pork colour between gilts and castrates from different crossbred pigs, whereas Newcom *et al*., (2004) reported lower yellowness in pork from gilts compared with castrates of purebred Duroc and Landrace pigs. Warris *et al*. (1990) reported higher EEL reflectance and more red pork colour in castrates than gilts from purebred Large White, Hampshire and Pietrain pigs.

Variations in pigment content between pork breeds have been reported. Loin from Hampshire pigs had higher pigment content compared with loin from Large White (Monin & Sellier, 1985) and Landrace pigs (Lindahl *et al*., 2001, Paper I) with Yorkshire being intermediate (Lindahl *et al*., 2001, Paper I). Newcom *et al*. (2004) reported loin pigment contents in the order Hampshire>Duroc>Yorkshire>

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Landrace purebred pigs. Duroc-sired pigs had higher loin pigment content than Landrace-sired pigs (Lindahl *et al*., 2005c, Paper III). Other studies showed no breed differences in pigment content (Lindahl *et al*., 2005d, Paper V).

Higher pigment content was found in muscles from gilts compared with castrates in Hampshire crossbreeds (Lindahl *et al*., 2005a, Paper VI) and in muscles from gilts compared with castrates, boars being intermediate of Large White × Landrace crossbreed (Warris *et al*., 1990). Barton-Gade (1987) did not find differences in pigment content between entire male, castrated male and female pigs of crossbreeds including Hampshire and Landrace. Likewise, Newcom *et al*. (2004) reported no difference in pigment content of LD between castrates and gilts of purebred Duroc, Hampshire, Landrace and Yorkshire pigs. Higher pigment content was found in LD from female pigs compared with entire male pigs in crossbreeds of Hampshire and Landrace (Lindahl *et al*., 2004b, Paper II) and in BF of gilts compared with castrates from Yorkshire pigs (Lindahl *et al*., 2001, Paper I).

Muscle

Muscles can be classified as glycolytic (white) or oxidative (red) due to their dominating energy metabolism, which is based on the proportion of glycolytic and oxidative fibre types in the muscle (Cassens *et al*., 1968; Beecher *et al*., 1965, 1969). Glycolytic muscles require a rapid source of energy, and glycolysis is the predominate metabolic pathway used by these muscles. Glycogen and many of the enzymes related to glycolysis are abundant in these muscles. Oxidative muscles rely primarily on oxidative metabolism, which requires a large amount of myoglobin for oxygen storage. As compared with white fibres, the red muscle fibres tend to be smaller in diameter, richer in mitochondria, myoglobin and lipid, and more generously supplied with blood due to higher capillarisation (Foegeding, Lanier & Hultin, 1996).

The proportion of different fibre types may vary markedly in different species and different muscles, depending on physiological function (Essén-Gustavsson, 1992). The variation in fibre types between species is caused by both genetic and environmental factors. There are marked differences in fibre types, fibre morphology and metabolic profiles between muscles both within and between pigs (Essén-Gustavsson, 1992). Among porcine muscles, inside part of BF is oxidative (Karlsson *et al*., 1993; Essén-Gustavsson *et al*., 1994), whereas LD (Kiessling & Hansson, 1983; Karlsson *et al*., 1993; Essén-Gustavsson *et al*., 1994) and *M. semimenbranosus* (SM) (Kiessling & Hansson, 1983) are glycolytic within the same breed. However, the fibre composition in a muscle may differ between breeds (Ruusunen & Poulanne, 1997), and the metabolic profile of a muscle with similar fibre type composition may differ between breeds (Essén-Gustavsson & Fjekner-Modig, 1985). The metabolic profile may also differ within a muscle. The inside part of BF was classified as red muscle with a high oxidative enzyme activity and the outside part of BF as white muscle with a low oxidative enzyme activity (Beecher *et al*., 1965). Furthermore, LD from carriers of the RN^- allele in Hampshire pigs is more oxidative and less glycolytic than non-carriers (Lebret *et al*., 1999).

The ultrastructure of glycolytic fibres differs between RN^- and rn^+ pigs (Estrade, Vignon & Monin, 1993). The glycolytic fibres of rn^+ pigs have narrow and regular intermyofibrillar spaces as usually observed in normal muscle, whereas the glycolytic fibres in muscle from RN^- pigs have an abnormally large intermyofibrillar space, broad and irregular in shape, and the section of the myofibrilles is very variable. In both cases, glycogen particles are abundant in all sarcoplasm, but at a much higher density in RN^- muscles, especially in glycolytic muscles.

The colour varies between pork muscles as a consequence of differences in pigment content and muscle metabolism (Brewer *et al*., 2001; Rosenwold & Andersen, 2003a; Lindahl, *et al*., 2001, paper I; Lindahl *et al*., 2005a, Paper VI).

Strategic feeding

Creatine

It has been suggested that increased creatine phosphate (CP) in the muscle may delay the lactate formation and consequently postpone the pH decline pm. CP plays an important role in maintaining the energy state in the muscle cell (Young & Young, 2002). Creatine taken up by skeletal muscle is phosphorylated by creatine kinase and functions to buffer changes in ATP during altered energy states (Connett, 1988; Meyer & Foley, 1996). The fraction of the total creatine pool that is in the form of CP serves as a marker of the energy state of the cell (Connet, 1988). Creatine, methylguanidine-acetic acid, is a naturally occurring tripeptide synthesised from arginine, glycine and methionine (Casey & Greenhaff, 2000). Most of the creatine pool in mammals is contained in skeletal muscle and approximately two thirds in the form of CP (Balsom, Söderlund & Ekblom, 1994; Casey, *et al*., 1996; Casey & Greenhaff, 2000).

Dietary supplementation of creatine has been shown to increase both CP and creatine levels in muscle of man (Harrris *et al*. 1992; Balsom, Söderlund & Hultman, 1995), rat (Young & Young, 2002; Ceddia & Sweeney, 2004) and pig (Lindahl, *et al*., 2005d, paper V), although not in the same proportion (Young & Young, 2002). Changes in the CP/creatine ratio indicate an altered energy state in the cell. The rate of CP utilisation is extremely rapid during the initial seconds of high-energy exercise, when the primary function of CP is thought to be serving as a buffer to the delay in energy provision from glucogenolysis (Casey & Greenhaff, 2000). Availability of CP is crucial to ATP resynthesis and high-intensity exercise performance (Casey & Greenhaff, 2000).

CP is involved in oxidative ATP production in mitochondria, which is regulated by the availability of mitochondrial ADP (Bessman & Savabi, 1990; Walliman *et al*., 1992). ADP formation and ATP resynthesis are linked to the phosphorylation of free creatine at the mitochondrial membrane. Upon creatine supplementation, it might be possible that the rate of CP resynthesis from mitochondrial ATP accelerates due to an increased free creatine concentration in muscle that enhances the rate of the creatine kinase reaction at the mitochondrial membrane (Casey & Greenhaff, 2000). This hypothesis is, according to Casey & Greenhaff (2000), supported by studies showing that the increase in total muscle creatine

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concentration after creatine ingestion is principally in the form of free creatine (Harris *et al*., 1992; Greenhaff *et al*., 1994). In vitro studies have shown that creatine can be used to increase the rate of respiration in skeletal muscle mitochondria (Bessman & Fonyo, 1966) and skinned cardiac muscle fibres (Field *et al*., 1994). Furthermore, creatine supplementation is shown to shift the basal glucose metabolism towards oxidation and reduced lactate production in L6 rat skeletal muscle cells (Ceddia & Sweeney, 2004), in mitochondria isolated from *i*) muscles of creatine-supplemented human subjects (Walsh *et al*., 2001) and *ii*) skinned cardiac muscle fibres from mice incubated with creatine (Saks *et al*., 2000).

The effect of dietary creatine is linked to the uptake in the muscle, which is influenced by the total creatine concentration before supplementation (Casey & Greenhaff, 2000). In general, the lower initial creatine concentration, the greater extent of muscle creatine uptake. The creatine uptake in muscle is also influenced by pig breed, with higher uptake in Duroc LD than in Landrace LD (Lindahl, 2005d, paper V).

Casey & Greenhaff (2000) indicate a muscle-specific effect of creatine supplementation, with greater effect in human type II than in type I fibres. Muscle tissue with a high concentration of type IIB fibres has the potential for a rapid pH decline *post mortem*, while the carcass temperature remains high, which could result in protein denaturation and a reduction in meat quality (Berg & Allee, 2001). Muscles with a high percent of type IIB fibres, such as LD and SM (Essén-Gustavsson, Karlström & Lundström, 1992), might therefore benefit from increased muscle creatine content delaying the formation of lactic acid.

Strategic feeding of pigs with creatine monohydrate (CMH) during a period of 5 to 15 days immediately before slaughter has shown to affect pork colour (Stahl, Alle & Berg, 2001; Maddock *et al*., 2002; Young *et al*, 2005; Lindahl *et al*, 2005d; paper V). However, some other studies showed no effect on pork colour of 10 days (O'Quinn *et al*, 2000, Berg & Allee, 2001) or 30 days of supplementation (Stahl & Berg, 2003). Stahl, Allee & Berg (2001) reported that dietary CMH increased the L* value and decreased the a* value in *M. gluteus medius* (GM), although no effect on ultimate pH was found. Furthermore, the L* value in LD was increased at 24 h pm, and an ageing period of 7 days pm increased the b* value and deceased the a* value. Maddock *et al*. (2002) found lower L* value in ham muscles from halothane gene carriers of a Duroc crossbreed supplemented with CMH, in line with the higher pH_{45} and tendency to higher pH_{24h}. Young *et al*. (2005) reported lower a* value in LD as an effect of dietary CMH in Duroc and Landrace pigs.

Vitamin E

Vitamin E supplementation has been used successfully to improve the colour stability of fresh beef. In contrast, the results obtained using the same strategy in relation to colour stability of pork have been inconclusive (Faustman & Wang, 2000). Some studies have shown improved colour stability after vitamin E supplementation, whereas several studies have shown no effect on pork reviewed by Jensen, Lauridsen & Bertelsen, (1998) and Rosenvold & Andersen (2003b) and

also more recent studies show limited effect on pork colour (Hasty *et al.*, 2002; O'Sullivan *et al.*, 2002; Geesink *et al.*, 2004; Swigert *et al.*, 2004).

The mechanism by which endogenous vitamin E improves colour stability is not clear, but discolouration and lipid oxidation in fresh meat are closely related (Jensen *et al.*, 1998). Furthermore, the activity of MetMb-reducing systems is believed to be retained for longer periods in meats with reduced lipid oxidation. As mentioned above, Lynch *et al.* (1998) suggest that vitamin E enhances MetMb reduction. Rosenvold *et al.* (2002) suggest that vitamin E may directly affect the mechanisms controlling glycogen metabolism. Vitamin E may thus influence early *post mortem* pH decline, which affects the colour of pork (Warris & Brown , 1987: Bendall & Swatland, 1988; Lindahl *et al.*, 2001, Paper I; Støier *et al.*, 2001; Rosenvold & Andersen, 2003a; Lindahl *et al.* 2004b, Paper II; Lindahl *et al.*, 2005a,b,d, Papers VI, IV, V).

Vitamin D_3

Dietary supplementation with vitamin D_3 was shown to affect pork colour (Wilburn *et al.*, 2004). Their hypothesis was that vitamin D_3 would shift muscle metabolism to be more oxidative and hereby decrease the rate and extent of pH decline, thus improving colour. However, Swigert *et al.* (2004) found no effect of dietary supplementation with vitamin D_3 on pork colour.

Magnesium

Dietary supplementation with magnesium has been shown to reduce the effect of stress before slaughter and thus decrease the incidence of PSE (D'Souza *et al.*, 1998, 1999) and improve the colour of pork (D'Souza *et al.*, 1998, 1999, 2000; Hamilton *et al.*, 2002, 2003). However, other studies failed to show any effect on the colour of pork (Geesink *et al.*, 2004; Swigert *et al.*, 2004).

Pre-slaughter handling and slaughter process

Stress and stunning

Stress prior to slaughter and stunning method influences stores of glycogen and energy metabolites such as CP (Støier *et al.*, 2001; Lindahl *et al.*, 2005b, Paper IV) and ATP (Lindahl *et al.*, 2005b, Paper IV), muscle temperature early *post mortem* (Brown *et al.*, 1998; van der Wal *et al.*, 1999; Hambrech, Eissen & Verstegen, 2003; Rosenvold & Andersen, 2003a; Hambrecht *et al.*, 2004ab; Lindahl *et al.*, 2005b, Paper IV), pH decline *post mortem* (Milligan *et al.*, 1998; van der Wal *et al.*, 1999; Hambrecht, Eissen & Verstegen, 2003; Rosenvold & Andersen, 2003a; Hambrecht *et al.*, 2004a,b; Lindahl *et al.*, 2005b, Paper IV), ultimate pH (Støier *et al.*, 2001; Hambrecht, Eissen & Verstegen, 2003; Hambrecht *et al.*, 2004b), internal reflectance (FOP) (Støier *et al.*, 2001; Hambrecht *et al.*, 2004ab) and pork colour (van der Wal, Engel & Hulsegge, 1997; Milligan *et al.*, 1998; Channon, Payne & Warner, 2000, Støier *et al.*, 2001; Hambrecht, Eissen & Verstegen, 2003; Rosenvold & Andersen, 2003a; Hambrecht *et al.*, 2004a,b; Hambrecht & Eissen, 2004; Lindahl *et al.*, 2005b,

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Paper IV). Finally, electrical stunning is shown to induce lighter colour compared with CO_2-stunning (Verlade *et al*., 2000; Channon, Payne & Warner, 2002).

Generally, more stressful conditions induce higher muscle temperature and lower pH early *post mortem* with or without higher ultimate pH (Schäfer *et al*., 2002). These metabolic changes *post mortem* are shown to have diverging influence on colour such as lighter (Milligan *et al*., 1998; Hambrecht *et al*., 2004ab; Hambrecht & Eissen, 2004; Lindahl *et al*., 2005b, Paper IV) or darker (Hambrecht & Eissen, 2004), more red (Milligan *et al*., 1998; Rosenvold & Andersen, 2003a; Hambrecht & Eissen, 2004; Lindahl *et al*., 2005b, Paper IV) or less red (Rosenvold & Andersen, 2003a; Hambrecht & Eissen, 2004), more yellow (Milligan *et al*., 1998; Rosenvold & Andersen, 2003a; Lindahl *et al*., 2005b, Paper IV) or less yellow (Rosenvold & Andersen, 2003a), higher FOP (Støier *et al*., 2001; Hambrecht *et al*., 2004a,b) or no effect depending on pig breed, type of stress, slaughter procedure and muscle studied. Stress had no influence on colour stability during storage (Milligan *et al*., 1998; Rosenvold & Andersen, 2003a).

Muscle temperature and cooling

Exercise and increased pre-slaughter and early *post mortem* muscle temperature result in lower glycogen and CP contents and higher creatine and lactate contents at slaughter followed by increased metabolite breakdown *post mortem* and slightly lighter colour (Klont, Talman & Monin, 1994; Klont & Lambooy, 1995ab).

The cooling rate of the pig carcass or excised muscles after slaughter influences pH decline and ultimate pork colour. Superficial muscles like LD are cooled more rapidly than interior muscles like SM (Hambrecht *et al*., 2004a; Lindahl *et al*, 2005a, Paper VI). Rapid cooling decreases the rate of pH decline (Jones *et al*, 1991; Jones *et al*, 1993; McCaw *et al*., 1997; Milligan *et al*, 1998; Springer *et al*., 2003; Hambrecht *et al*., 2004a; Lindahl, *et al*., 2005d, Paper V) either with slightly increased ultimate pH (Jones *et al*, 1993) or no effect on ultimate pH (Jones *et al*, 1991; Springer *et al*., 2003; Lindahl, *et al*., 2005d, Paper V). A slight decrease of 1 to 2°C early *post mortem* influences the rate of pH decline as well (Maribo *et al*., 1998). Several studies showed that fast cooling, with a consequent slower pH decline, resulted in darker (Jones, Tong & Murray, 1987); Jones, Jeremiah & Robertson, 1993; McCaw *et al*., 1997; Springer *et al*., 2003; Lindahl, *et al*., 2005d; Paper V), less red (Jones, Jeremiah & Robertson, 1993; Lindahl, *et al*., 2005d, Paper V) and yellow colour (Jones, Jeremiah & Robertson, 1993; McCaw *et al*., 1997; Ohene-Adjei *et al*., 2002; Springer *et al*., 2003; Lindahl, *et al*., 2005d, Paper V), although Milligan *et al*. (1998) found lighter colour. Other studies showed no effect of cooling rate on pork colour despite effect on pH decline (Jones *et al*, 1991; Hambrecht *et al*., 2004a).

Pork colour related to pH

The pH decline and ultimate pH influence meat quality in terms of colour and water-holding capacity. Dark, firm, dry (DFD) and pale, soft, exudative (PSE) meats were the first recognised meat quality defects and later red, soft and exudative (RSE) meat (Joo *et al*., 1995) and pale, firm and non-exudative (PFN) meat (van Laack *et al*., 1994) have been characterised. Normal meat is also

referred to as red, firm and nonexudative (RFN) (van Laack *et al*., 1994; Joo *et al*., 1995). The classification of meat in the different quality groups is based on ultimate pH, water-holding capacity measured as percentage drip loss during 48 hours and lightness (*L** value). The limits for the different quality groups vary between different studies (van Laack *et al*., 1994; Joo *et al*., 1995; Joo *et al*., 1999; van Laack & Kauffman, 1999). The numerical values of the colour parameters *L**, *a** and *b** depend on the instrument used (Baardseth *et al*., 1988; Brewer *et al*., 2001), and thus the limit for lightness has to be settled with the actual instrument. Characteristics of the pork qualities are shown in Table 2.

Table 2. *Characteristics of different pork qualities*

Quality	Colour[1]	*L** value[1]	Drip %[1]	pH
DFD	Dark	≤52	<5.0	High ultimate (>6.0)
RFN	Red	52-58	<5.0	Normal
PFN	Pale	≥58	<5.0	
RSE	Red	52-58	≥5.0	
PSE	Pale	≥58	≥5.0	Fast pH decline

[1]According to van Laack *et al*. (1994)

DFD meat is characterised by high pH (> 6.0), dark colour and very little drip. When pH is much above the isoelectric point of actomyosin, more water is kept between the myofilaments, resulting in a subsequent decrease of the fluid phase of extramyofibrillar spaces of the muscle. The muscle has a compact structure and appears darker in colour, since its surface only scatters incident light to a very small extent (Govindarajan, 1973). In addition, at these high pH values, the oxygen-consuming enzymes present in the meat are relatively active so that little surface oxygenation of the myoglobin occurs, with the result that the purple, reduced pigment Mb dominates (Govindarajan, 1973; Egberth & Cornforth, 1986). Colour measurements of DFD pork compared with normal pork have shown lower lightness and yellowness (van der Wal *et al*., 1988; Joo *et al*., 1995; Joo *et al*. 1999; Lindahl, unpublished data) and lower (Joo *et al*., 1995; Joo *et al*. 1999; Lindahl, unpublished data) or slightly higher redness (van der Wal *et al*., 1988). However, Zhu & Brewer (1998b) reported no differences in lightness and redness between DFD and normal pork. The differentiation between DFD and normal meat in the above studies was based on pH with different limits and on visual colour evaluation. It is important to use the same classification when comparing results from different studies, as the colour is highly dependent on the pH of the meat. Zhu & Brewer (1998a) found higher MetMb reductase activity and oxygen consumption rate in DFD compared with normal pork classified by visual colour evaluation.

At normal ultimate pH values (approx. 5.5) in the meat, the fibres hold less water, and the oxygen-consuming enzymes are less active resulting in a brighter, glossier appearance (Ledward, 1992). This situation prevails if the fall in pH, from *in vivo* value 7.3 to the ultimate pH, occurs at chill temperatures (Ledward, 1992). The pH of normal meat is much closer to the isoelectric point of myosin. This leads to the loss of water-binding capacity of the protein and a structure with an

increased extra myofibrillar space. Since the muscle is not densely packed, more incident light is scattered at the surface and hence it has a lighter colour (Govindarajan, 1973; Offer *et al.*, 1989). Inactivation of the mitochondria due to low pH in normal meat inhibits the oxygen consumption, thereby allowing myoglobin to be oxygenated, which results in the bright red colour of MbO_2 (Govindarajan, 1973). If, however, the low pH is achieved at higher temperatures, then some partial denaturation of myosin and of sarcoplasmic proteins may occur, so that the water-holding capacity is decreased still further (Ledward, 1992), and light scattering is increased (Bendall & Swatland, 1988) resulting in PSE meat. Colour measurements of PSE pork compared with normal pork showed higher lightness, redness and yellowness (van der Wal *et al.*, 1988; Joo *et al.*, 1995; Joo *et al.*, 1999) in PSE pork. Zhu & Brewer (1998b) found higher lightness and lower redness in PSE pork compared with normal pork. As mentioned above, it is important to use the same classification when comparing results from different studies. Zhu & Brewer (1998a) found lower MetMb reductase activity, but no difference in oxygen consumption rate in PSE pork compared with normal pork classified by visual colour evaluation.

The incidence of RSE pork has been studied and discussed in relation to the frequency of the RN^- gene carriers of Hampshire crossbred pigs (van Laack & Kauffman, 1999; Bertram, Petersen & Andersen, 2000). van Laack & Kauffman (1999) found only 10% of the RSE samples being RN^- carriers, concluding that the occurrence of RSE quality is not related to the presence of the RN^- gene. Bertram, Petersen & Andersen (2000) found RSE-like pork within both RN^- carriers and non-carriers, concluding that the presence of RN^- gene only partly explains the variation in drip loss.

Effect of storage on pork colour

Anaerobic storage

Myoglobin is preserved in its reduced deoxygenated form, Mb, when meat is chill-stored without exposure to air, i.e. in a vacuum package. The colour does not change during storage as long as the package is intact, and oxygen is totally excluded. However, storage time under anaerobic conditions influences the colour when the meat is exposed to air. Ageing increases the ability of pork to bloom (Zhu, Bidner & Brewer, 2001; Lindahl *et al.*, 2005a,c, Papers VI, III), resulting in increased lightness, redness and yellowness (Apple *et al.*, 2001; Zhu *et al.*, 2001; Lindahl *et al.*, 2005a,c Papers VI, III) or increased redness and yellowness (Rosenvold & Andersen, 2003a). On the contrary, Frederick, van Heugten & See (2004) reported decreased lightness, and Schluter *et al.* (2001) decreased yellowness upon storage. Apple *et al.* (2002) found increased lightness after vacuum storage of pork loin from both halothane negative pigs and halothane gene carriers, but increased redness and decreased yellowness was only found in loin from halothane gene carriers. The lighter, redder and more yellow colour is caused by reduced oxygen consumption potential as a function of time *pm* hereby allowing more rapid blooming (Atkinson & Follet, 1973).

Aerobic storage

Colour measured 24 hours *post mortem* is generally included in the assessment of pork quality, but colour stability during retail display is more seldomly studied. Furthermore, there are few studies on the effect of ageing on colour stability of pork. Colour changes during air-exposed storage of pork are mainly related to oxidation of myoglobin to MetMb (Lindahl *et al*., 2005a,c, Papers VI, III). However, the colour changes during the first storage day are related to oxygenation of Mb to MbO_2 (blooming), if the pork is cut during the first days *post mortem* and not aged before exposure to air (Lindahl *et al*., 2005a,c, Papers VI, III). This blooming of pork during the first storage day with air-exposure results in increased lightness, redness and yellowness (Lindahl *et al*., 2005a,c, Papers VI, III) as discussed above. The accumulation of MetMb on the surface of pork upon further storage affects redness, which decreases with storage time (Rosenvold & Andersen, 2003a; Lindahl *et al*., 2005a,d, Papers VI, V). Lightness tends to increase with storage time, whereas yellowness remains constant (Rosenvold & Andersen, 2003a; Lindahl *et al*., 2005a,d, Papers VI, V). No effect of ageing time *post mortem* on the oxidation myoglobin to MetMb was found in LD from Landrace- or Duroc-sired pigs (Lindahl *et al*., 2005c, Paper III), whereas ageing tended to increase oxidation in LD and SM from the rn^+ genotype of Hampshire crossbred pigs (Lindahl *et al*., 2005a, Paper VI). Rosenvold & Andersen (2003a) reported faster decrease in redness in aged compared with not aged pork. Higher levels of MetMb in aged pork cold be due to loss of MetMb-reducing activity (Zhu & Brewer, 1998a) and a combination of depletion of required substrates and co-factors (Giddings, 1974) and loss of mitochondrial structure (Cheah & Cheah, 1971; Tang *et al*, 2005).

Objectives

The objective of the present thesis was to obtain more fundamental understanding of the mechanisms for variations in colour and colour stability of fresh pork. The effects of breed and genotype, strategic feeding with creatine, pre-slaughter treatment, slaughter procedure and carcass cooling on ultimate colour and colour stability were studied. The specific aims of this thesis were to obtain more understanding concerning the following questions:

- *Contribution of pigment content, myoglobin species and internal reflectance to the colour of fresh pork* (Paper I). These factors are known to influence meat colour, but information on the contribution of each factor to the colour of pork was lacking. Multivariate methods were used to study interactions and relative importance of the single factors.

- *Influence of early post mortem progress in temperature and pH on the colour and colour stability of fresh pork.* (Papers II, III, IV, V & VI). The colour of meat is highly affected by pH with DFD and PSE meat as extremes, but also more moderate variations in pH decline and ultimate pH influence the colour of pork. It has been suggested that increased creatine phosphate in porcine muscle may delay lactate formation and consequently postpone the pH decline. The effects of early *post mortem* temperature and pH progress on the variation in the colour of pork in terms of tristimulus chromatic attributes, myoglobin species and internal reflectance were studied. Dietary supplementation with creatine monohydrate (CMH), pre-slaughter stress and variations in genetic background and carcass cooling rate were used to induce differences in early *post mortem* temperature and pH progress.

- *Significance of pig breed for the colour and colour stability of fresh pork.* (Papers I, II, III, IV, V & VI). The colour of pork varies with the genetic background of the pigs. The reason for this variation is not fully understood, and information about the effect of genotype on colour stability is scarce. Pork from Duroc, Hampshire, Landrace and Yorkshire pigs, pure breeds and crossbreeds were used to investigate the underlying factors for variation in colour and colour stability in terms of tristimulus chromatic attributes, pigment content, myoglobin species and internal reflectance.

- *Significance of the RN genotype for the colour and colour stability of fresh pork.* (Papers II, IV & VI). The *RN* genotype is known to influence pork colour. Hampshire crossbreeds with three different alleles on the *RN* locus (RN^-, rn^+ and $rn*$ [V199I]) were used to investigate the underlying factors for the genotypic variation in colour and colour stability in terms of tristimulus chromatic attributes, pigment content, myoglobin species and internal reflectance.

Materials and Methods

Animal material and sampling

Pork from pure bred and crossbred pigs were used in the different studies according to Table 3.

Table 3. *Pig breeds used in the studies presented in papers I-VI. D=Duroc; H=Hampshire; L=Landrace; Y=Yorkshire*

Breed	Paper	Sire×Dam
Purebreeds	I	H×H; L×L; Y×Y
	V	D×D; L×L
Crossbreeds	II	LH×LH[1]; H×LH[1]; LH×H[1]
	III	D×LYD; L×LYD
	IV	D×LY[2]; H×LY[3]
	VI	H×LY

[1]*RN⁻, rn⁺, rn** genotypes; [2]*NN* and *nn* genotypes; [3]*RN⁻* and *rn⁺* genotypes

Paper I

LD and BF samples from 96 pigs of three pure breeds (32/breed; Table 3), females and castrates, were used. The pigs were slaughtered at the same live weights (96 to 116 kg) for each breed. All muscles were judged to be of normal meat quality (no PSE or DFD meat) by visual inspection, pH and Fibre Optic Probe (FOP) measurements.

Three days *pm*, 2 cm slices of LD and BF, were cut across the fibre direction just in front of the last rib in LD and on the middle deep part of BF. The slices of meat were wrapped with oxygen permeable film and stored at 4°C for 1.5 to 2 hours prior to colour measurements. FOP and pH measurements and samples for analysis of pigment content were taken in 4 cm slices of LD and BF next to those for colour measurements.

Paper II

LD samples from 334 pigs of three crossbreeds (Table 3), entire males and females were used. The pigs were raised at three breeding farms, but slaughtered at the same commercial slaughterhouse over a period of 11 months. The stunning procedure was changed from individual stunning with CO_2 (96 to 97% CO_2 for 1.5 min; 223 pigs) to group stunning with CO_2 (96 to 97% CO_2 for 2 min) in groups of five (111 pigs) during the slaughter period.

Measurements of pH and FOP were taken in LD at the last rib 24 h and 3 days *pm*. Samples for analysis of pigment content were taken in LD next to the measurements of pH and FOP. Three days *pm*, 2 cm slices of LD were cut across the fibre direction, wrapped with oxygen permeable film and stored at 4°C for 1.5 to 2 hours prior to colour measurements.

Paper III

LD samples from 12 Duroc-sired and 19 Landrace-sired (Table 3) female and castrated male pigs were used. The pigs were slaughtered at approximately 105 kg live weight in the experimental abattoir at Research Centre Foulum. The pigs were stunned with 80% CO_2 for 3 min, exsanguinated, scalded at 62°C for 3 min, cleaned and eviscerated within 30 min and chilled at 3°C after 60 min.

LD was excised from the carcasses 24 hours *pm*. The muscle was divided into 2 parts, and one part was vacuum-packed and stored at 3°C for another 7 days (= 8 days of ageing) prior to colour measurements. The other part of the muscle was used directly for colour measurements (= 1 day of ageing). Slices of LD, 2 cm thick, were cut across the fibre direction, wrapped with oxygen permeable film and stored at 3°C for 6 days. Samples, 10 cm LD cut just behind the last rib, were taken for analysis of dry matter, intramuscular fat (IMF) and protein.

Paper IV

LD samples from 181 pigs from two crossbreeds (Table 3), females and castrated males, were used. Two treatments were applied to obtain variations in the metabolic processes *pm*. One group of pigs (Control) was exposed to minimum stress prior to slaughter and was at slaughter stunned by 80% CO_2 for 3 min: 23 $DDLY_{NN}$, 18 $DDLY_{Nn}$, 33 $HDLY_{rn+}$ and 19 $HDLY_{RN-}$. The other group (ExEl) was exercised immediately prior to stunning on a treadmill till exhaustion (breathing and stride frequency become uncoordinated) and then electrically stunned (220V, 1.5 A, 15 sec): 19 $DDLY_{NN}$, 21 $DDLY_{Nn}$, 25 $HDLY_{rn+}$ and 23 $HDLY_{RN-}$. All pigs were exsanguinated after stunning, scalded at 62°C for 3 minutes, and then cleaned and eviscerated within 30 minutes. The carcasses were stored in a chilling room at 4°C after 60 minutes.

Biopsies for analysis of metabolites were taken just above the last rib immediately prior to stunning and at 1 min, 15 min, 30 min, 1 hours, 2 hours and 24 hours after bleeding. In the live animals, biopsies were taken with a spring-loaded biopsy instrument (Biotech PPB-U, Slovakia) immediately prior to stunning. Muscle sampling was in compliance with Danish regulations for the humane care and use of animals in research, and the protocols applied were approved by the Danish Animal Experiments Inspectorate. *Pm* biopsies were taken with a modified form of the Bergström needle (Bergström, 1962), with an outer diameter of 6 mm. Immediately following excision, the samples were frozen in liquid nitrogen and stored at −80°C until further analysis.

Both loins were excised from the carcass at 24 h *pm*, and 2 cm thick loin chops were cut at 2 cm from the last rib in the cranical direction. The chops were bloomed for 1 h at 4°C prior to colour measurement The colour measurements were performed on the right and left side of the carcasses, and mean values from both sides were used for statistical analysis.

Paper V

LD samples from 36 female pigs of two pure breeds (18/breed; Table 3) were used. Five days before slaughter the pigs were allocated to two treatment groups, basal diet supplemented with 0 or 50 g creatine monohydrate (CMH)/d during 5 days. The pigs were slaughtered at the experimental slaughter plant at the Danish Institute of Agricultural Sciences, Research Centre Foulum. Pigs were stunned by 80% CO_2 for 3 min, exsanguinated, scalded at 62°C for 3 min, cleaned, and eviscerated within 30 min. At 45 min *pm* the carcasses were placed at approximately 13°C, and at 1 h *pm* one half of the carcass (left and right randomised) was placed in a chilling room at 4°C (slow cooling) and the other half in a freezer for 1 h at approximately –28°C (fast cooling), and then transferred to a separate chilling room at 4°C. The experiment was performed in 9 replicates including 2 treatments x 2 breeds x 2 cooling rates.

Biopsies for analysis of phosphorous compounds (CP and ATP) were taken in LD at the time of exsanguination. At 24 h *pm*, 2 cm thick chops of LD were cut across the fibre direction at 2 cm from the last rib in the caudal direction for colour measurements. The chops were wrapped with oxygen permeable film and stored at 3°C for 6 days. Samples (100 mg) for analysis of pigment content were taken from the chop opposite the one for colour measurements.

PaperVI

LD and SM from 29 crossbred pigs (Table 3), female and castrated males were used. The pigs were slaughtered at a commercial slaughterhouse using CO_2-stunning, and the carcasses were exsanguinated, scalded, eviscerated and split within 30 min *pm*. After 45 min the carcasses were chilled at 4°C until they at approximately 1.5 h *pm* were transferred to –17°C for one h and subsequently stored in a chilling room at 4°C.

LD and SM were excised from the carcasses 48 hours *pm*. Both muscles were divided into 2 parts, and one part was vacuum-packed and stored at 5°C for another 7 days (= 9 days of ageing) prior to colour measurements. The other parts of the muscles were used directly for colour measurements (= 2 days of ageing). Slices of LD and SM, 2 cm thick, were cut across the fibre direction, wrapped with oxygen permeable film and stored at 5°C for 6 days for analysis of colour stability.

Methods

Measurements of pH, temperature and internal reflectance

Papers I & II

Measurements of pH (Knick Portamess 651, Xerolyte electrode (W. Ingold Ltd, Urdorf, Switzerland) and internal reflectance (FOP; Fibre Optic Probe, MkIII; TBL Fibre Optic Groups, Leeds, UK) were taken on slices of LD (Papers I-II) and BF (Paper I) cut next to the slices for colour measurements 24 hours (Paper II) and 3 days *post mortem* (Paper I-II).

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Paper III

Temperature (Testo 110 thermometer, Testo Gmbh 6 Co, Germany) and pH (PHM201 pH Meter, Radiometer, Denmark, equipped with Methrom LL combined pH penetration electrode (Methrohm, Switzerland) were measured in LD at the last rib 1 min, 45 min and 24 hours pm. The pH electrode was calibrated in pH 4.01 and 7.00 buffers equilibrated at 35°C for the measurements on the warm carcass 1 min and 45 min pm, and at 4°C for the measurement on the cold carcass 24 hours pm.

Paper IV

Duplicate pH measurements were taken at the last thoracic vertebra with a pH-meter (Radiometer, Denmark) equipped with an insertion glass electrode (Metrohm, Switzerland) at the following fixed intervals *pm*: 1 min, 15 min, 30 min, 1 hour, 2 hours and 24 hours. Temperature was measured at the same position on the muscle at the same time intervals with a Testo 110 insertion thermo-element (Testo, Germany). The pH electrode was calibrated in pH 4.01 and 7.00 buffers (Radiometer, Denmark) at 35°C, when pH was measured 1 min to 2 h *pm*, and at 4°C, when pH was measured 24 h *pm*. The pH and temperature measurements were taken on the right and left side of the carcasses, and mean values from both sides were used for statistical analysis.

Paper V

Temperature and pH were measured in the loin at the level of the last rib. Temperature was logged every min from 20 min until 24 h *pm* with temperature loggers (StowAway TidbiT, Bourne, MA, USA). Muscle temperatures measured at 30 min, 45 min, 60 min and then taken with 30 min intervals up to 6 hours *pm* were included in the statistical analysis. Measurements of pH were carried out at 1, 15, 30, 45, 60, 120 and 1440 min (24 h) *pm* with a pH-meter (Radiometer PHM210, Copenhagen, Denmark) equipped with an insertion glass electrode (Model 704, Metrohm, Herisau, Switzerland) calibrated in buffers at pH 4.01 and 7.00 (Radiometer, Copenhagen, Denmark) at 35°C for all measurements up to 1 h *pm* and up to 2 h *pm* for slow-cooled carcasses. In fast-cooled carcasses, pH calibration was performed at ambient temperature in the period 1 h to 2 h *pm*, while pH calibration for measurements carried out 24 h *pm* and thereafter was performed at 4°C. The average of two measurements was used.

Paper VI

Temperature (MULTI, digital Thermometer) and pH measurements (Knick portable pH-meter equipped with a combination gel electrode, SE104, Knick, Berlin, Germany) were taken at the last thoracic vertebra in LD and in the middle of SM at the following intervals *pm*: 30 min, 3, 5, 24 and 48 h. The pH electrode was calibrated in pH 4.01 and 7.00 buffers (Radiometer, Denmark) at ambient temperature when pH was measured 30 min *pm*, and at 4°C at the other time points *pm*.

Determination of genotypes

Three RN alleles, RN^-, rn^+ and V199I (here denoted rn^*), were identified in muscle samples of LD according to Milan *et al*. (2000). The genotypes RN^-/RN^-, RN^-/rn^+, RN^-/rn^*, rn^+/rn^+, rn^+/rn^* or rn^*/rn^* were identified in the study presented in Paper II, but pigs of the rn^+/rn^* and rn^*/rn^* genotypes were studied as one genotype group denoted $rn^+/rn^*/rn^*$ due two the low number of pigs of each genotype.

Only two genotype groups, carriers (RN^- genotype) and non-carriers (rn^+ genotype) of the RN^- allele, were used in the study presented in Paper VI due to the low number of pigs of each genotype. The RN^- genotype included 7 RN^-/rn^+ and 4 RN^-/rn* pigs (6 castrates and 5 females), and the rn^+ genotype included 4 rn^+/rn^+, 11 rn^+/rn^* and 3 rn^*/rn^* pigs (10 castrates and 8 females).

The Hampshire crossings were differentiated in carriers and non-carriers of the RN^- allele based on a bimodal distribution of *peri mortem* glycogen content, and halothane genotype was determined with the method of Fujii *et al*. (1991) in the study presented in Paper IV

Carcass and compositional traits

Carcass weight

The weight of the left half of the cold carcass was registered 24 hours *post mortem* (Paper II). Warm carcass weight was registered by weighing the eviscerated carcass (minus tongue, bristles, genitals, kidneys, diaphragm and front feet) 45 min pm. Lean meat content was estimated from measurements using Fat-O-Meter (SFK Ltd, Hvidovre, Denmark) on the cold carcass (Kempster, Chadwick & Jones, 1985) (Paper III).

Pigment content

In the studies presented in Papers I & II, pigment content was analysed according to the method of Hornsey (1956), using a standard curve of haematin instead of the factor used by Hornsey (1956) and expressed as haematin mg/kg wet tissue. In the studies reported in Papers IV, V & VI, the pigment content was analysed as described by Oksbjerg *et al*. (2000) and expressed as mg myoglobin residues/g tissue. In the study described in Paper VI, the pigment content was analysed using the Nit409 method (Trout, 1991) with a minor modification. Sub-samples of 3 g of muscle were taken and homogenised with 30 ml of 0.04 M phosphate buffer using a Sorbent blender for 30s (at the speed of 6 on a scale from 1 – 10). The samples were kept on ice and centrifuged at 6000 rpm and 4°C for 15 min in an IEC PR7000 centrifuge. After centrifugation, 4 ml of each sample was mixed with 1.4 ml 0.15 M TritonX-100 solution and 100 µl 0.065 M sodium-nitrite solution, and the samples were shaken and kept at room temperature (20°C) for 1 hour. The amount of oxidised pigment was measured as metmyoglobin, at a wavelength of 410 nm using a spectrophotometer Unicam Spectronic UV 300. The pigment content was expressed as mg myoglobin residues/g wet tissue.

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Dry matter

Dry matter was analysed by drying the meat sample for 24 h at 103°C (Paper III).

Intramuscular fat (IMF)

IMF was analysed according to NMKL (1974) in the study presented in Paper I and according to NMKL (1989) in the study presented in Paper III.

Protein

Protein was analysed according to NMKL (1976) in the study presented in Paper III.

Glycogen

Glycogen was analysed as described by Henckel *et al.* (2002) in the study presented in Paper IV.

CP and ATP

The phosphorous compounds CP and ATP were analysed by high-performance liquid chromatography (HPLC) according to Henckel *et al.* (2002) (Paper IV), except the sample size and extraction volume were increased five times (Paper V).

Colour measurements

Papers I & II

The colour was measured using a Hunterlab Color Quest Instrument (Hunter Associates Laboratory, Inc., Reston, VA 22090 USA) with specular reflectance excluded, 25 mm measuring aperture, illuminant D_{65}, 10° Standard Observer and CIE (1976) colour scale. The average of 4 measurements across the surface was used. Chroma (saturation) was calculated as $(a^{*2} + b^{*2})^{1/2}$ and hue angle as arctan b^*/a^* (Wyszecki & Stiles, 1982). The fractions of Mb, MbO_2 and MetMb were calculated from the reflectance curve according to Krzywicki (1979) using 710 nm (the highest wavelength of the instrument) instead of 730 nm. The instrument measures the reflectance between 400 nm and 710 nm at 10 nm intervals. Reflectance values at wavelengths not given by the instrument (473, 525 and 572 nm) were calculated using linear interpolation.

Papers III, V & VI

The colour was measured using Minolta CM-2600d spectrophotometer (Minolta Co, Ltd, Osaka, Japan) with specular reflectance excluded, 8 mm diameter measuring aperture, illuminant D65, 10° Standard Observer and CIE (1976) colour scale. The measuring aperture was covered with a glass plate, and the instrument was calibrated against a white plate (L* = 97.30, a* = -0.06 b* = 0.00). The average of 4 measurements across the surface was used. The instrument measures reflectance between 400 and 740 nm at 10 nm intervals, and Kubelka-Munk K/S ratios were calculated using SpectraMagic ver. 3.6 software (Minolta Co, Ltd, Osaka, Japan). The relative content of Mb was estimated by the ratio $K/S_{474/525}$, the relative content of MbO_2 by the ratio $K/S_{610/525}$ and the relative content MetMb by

the ratio $K/S_{572/525}$ (Hunt *et al.*, 1991; Mancini, Hunt & Kropf, 2003). These ratios decrease when the relative content of the corresponding myoglobin species increases. The K/S-ratios were therefore multiplied by -1 in the diagrams (Fig. 2 and 4) in order to get the right impression when looking at the curves. K/S-ratios at wavelengths not given by the instrument (474, 525, 572 nm) were calculated using linear interpolation.

Blooming was assessed by colour measurements immediately after cutting (0 min) and after 10, 30, 60, 90 min and 24 h of blooming in air at 3°C (Paper III). Colour stability was assessed by colour measurements after 0 (= blooming for 60 min), 1, 2, 3 and 6 days of air-exposed storage at 3°C (Paper III), after 0 (= blooming for 1 hour), 1, 2 and 6 days at 4°C (Paper V) and after 0 (= blooming for 1 hour), 1, 2, 3, 4, 5 and 6 days at 5°C (Paper VI).

Paper IV

The colour was measured using a Minolta Chroma Meter CR300 (Osaka, Japan). A white tile (L*=93.30, a*=0.32 and b*=0.33) was used as standard. The averages of five measurements on each chop were used.

Analysis of variance

Paper I

Analysis of variance was performed on all the variables measured using the GLM procedure in SYSTAT 7.0 for Windows (SPSS Inc., USA). Tukey´s test was used for the analysis of significant differences between least squares means (LSM).

Paper II

Statistical evaluation was performed using the Procedure Mixed in SAS (Ver. 8e, SAS Institute Inc., Cary, NC, USA). Degrees of freedom were estimated with the Satterthwaite method, as the material was unbalanced. Significant differences between LSM were evaluated using the option Pdiff.

Papers III, IV, V, VI

Statistical analysis was carried out with the Statistical Analysis System version 8.02 (SAS Institute, Cary, NC, USA). The mixed procedure was applied when calculating LSM and standard errors SE. Degrees of freedom were estimated with the Satterthwaite method. Significant differences between LSM were evaluated using the option Pdiff.

Statistical model Paper I

Three different models were used; (1) Effect of sex within each breed: The model contained the effects of sire, dam within sire, and sex analysed for each breed separately. (2) Effect of breed: The model contained the effects of breed, sire within breed, dam within breed and sire, sex within breed. (3) Effect of muscle: The model contained the effects of breed, animal within breed and muscle within breed. The effect of animal was random, and all the other effects were fixed.

Statistical model Paper II

The model contained fixed effects of stunning procedure, genotype, crossbreed and sex, and random effects of herd and sire. Cold weight as a covariate and 2-way interactions were included when significant.

Statistical model Paper III

The statistical model included crossbreed, feeding regimen within crossbreed, ageing time pm, blooming or display time in air and their two-way interactions as fixed effects. Pig nested within crossbreed, feeding regimen and sex was included as random effects. The non-significant effect of feeding regimen was ignored in the final model. The GLM procedure was applied on carcass characteristics and meat composition using a model including crossbreed and sex as fixed effects.

Statistical model Paper IV

The model for colour parameters included fixed effects of crossbreed, genotype nested within crossbreed, treatment and gender. Two- and three-way interactions were included when significant. The model for temperature and pH included fixed effects of crossbreed, genotype nested within crossbreed, treatment, gender and time point *pm*. Two-way and three-way interactions were included when significant. The four-way interaction between crossbreed, genotype within breed, treatment and time point *pm* was included for calculation of LSM. Pig nested within breed, genotype, treatment and gender was included as random effect when repeated measurements were included.

Statistical model Paper V

For pH and temperature, the model included fixed effects of breed, dietary CMH, cooling rate and time point *pm*, random effects of replicate and pig nested within breed, replicate and dietary CMH. Two-way interactions were included when significant. Two-way interactions including time point *pm* were included, even if not significant, for calculation of LSM of pH at the different time points *pm*. The three-way interaction breed*cooling rate*time *pm* was included for calculation of LSM of temperature at the different time points *pm*. The same model with storage time after cutting the chops instead of time point *pm* was used for the colour characteristics. Two-way interactions including storage time were included, even if not significant, for calculation of LSM at the different storage times. A model including the fixed effects of breed and dietary CMH and their interaction and random effects of replicate and pig nested within breed, repetition and dietary CMH was used for myoglobin, CP and ATP contents.

Statistical model Paper VI

The models for temperature and pH included fixed effects of genotype (RN^- and rn^+), sex (female and castrate) and time *pm* (30 min, 3, 5, 24, 48 h) and random effect of herd and pig nested within herd. The model for colour characteristics included fixed effects of genotype, sex, ageing time *pm* (2 days and 9 days) and storage time (0 to 6 days) and random effects of herd and pig nested within herd. Two-way and three-way interactions were included in the models. A model

including the fixed effects of genotype and sex and random effect of herd was used for pigment content.

Multivariate analysis

Paper I

Principal Component Analysis (PCA) and Partial Least Squares Regression (PLS), were performed using The Unscrambler 6.11 (Camo AS, Trondheim, Norway) with standardised variables and random cross validation. Martens' uncertainty test was used for significance testing of the variables in PLS.

Paper II

The Unscrambler v7.6 SR-1 (Camo ASA, Oslo, Norway) was used for Principal Component Analysis (PCA) using standardised variables and random cross validation.

Paper IV

PLS was carried out with The Unscrambler version 9.1 (Camo Process AS, Oslo, Norway). PLS1 including pH at 1 min, 15 min, 30 min, 1 hour, 2 hours, 24 hours and temperature at 1 min, 15 min, 30 min, 1 hour and 2 hours *pm* as X-variables and L*, a* and b* values, respectively, as Y-variables were applied using, standardised variables, random cross validation and Martens' uncertainty test.

Paper V

PLS was carried out with The Unscrambler version 9.1 (Camo Process AS, Oslo, Norway). PLS1 including pH at 1 min, 15 min, 30 min, 45 min, 1 hour, 2 hours and 24 hours and muscle temperature at 30 min, 45 min, 60 min and then every 30 min up to 6 hours *pm* as X-variables and L*, a* and b* values, respectively, as Y-variables were applied using standardised variables, random cross validation and Martens' uncertainty test.

Summary of presented papers

I. Contribution of pigment content, myoglobin forms and internal reflectance to the colour of pork loin and ham from pure breed pigs

The colour of loin, *M. longissimus dorsi* (LD), and ham, *M. biceps femoris* (BF), from pure breed Hampshire (H), Swedish Landrace (SL) and Swedish Yorkshire (SY) pigs was studied. The contribution of the pigment content, the myoglobin species (forms) deoxymyoglobin (Mb), oxymyoglobin (MbO_2) and metmyoglobin (MetMb) and the internal reflectance to the colour of pork of normal meat quality was evaluated using partial least squares regression (PLS). The colour of LD and BF from the Hampshire breed was more red and yellow and more saturated than the colour of the same muscles from the Swedish Landrace and the Swedish Yorkshire breeds. Furthermore, BF from Hampshire was darker than BF from the other two breeds. These differences in colour were related to the higher pigment content and the lower pH in Hampshire, resulting in more blooming and higher internal reflectance. The colour of BF was darker and redder than the colour of LD within each breed. No colour difference was found between gilts and castrates within breeds. Most of the variation (86 to 90%) in lightness (L^* value), redness (a^* value) and yellowness (b^* value), chroma (saturation) and hue angle of pork of normal meat quality was explained by the pigment content, distribution in myoglobin species and internal reflectance. The L^* value, a^* value, chroma and hue angle were influenced by both the pigment content and by the distribution in myoglobin species to almost the same extent, while the internal reflectance was of no significance to these colour parameters. The b^* value was influenced most by the distribution in myoglobin species, less by the internal reflectance and almost not at all by the pigment content.

II. A second mutant allele (V199I) at the *PRKAG3* (*RN*) locus – II. Effect on colour characteristics of pork loin

Three alleles, RN^-, rn^+ and the new V199I (here denoted rn^*), at the *RN* locus in the *PRKAG3* gene that influences the glycogen content of pork have been identified in Hampshire × Landrace crossbred pigs, using a specific genotype test. The effect of these three alleles on ultimate pH, pigment content, internal reflectance (FOP), surface colour measured by tristimulus colorimetry (L^*, a^*, b^*) and fractions of deoxymyoglobin (Mb), oxymyoglobin (MbO_2) and metmyoglobin (MetMb) of pork loin was studied. Moreover, the effect of sex, entire male versus female pigs, on these traits was also included. The three alleles at the *RN* locus affected ultimate pH, internal reflectance, colour and distribution in myoglobin species of pork loin, but not the pigment content. The lowest ultimate pH was found in loin of the $RN^-/-$ genotypes, slightly higher pH in loin of the rn^+/rn^+ genotype and the highest pH in loin of the rn^+/rn^* and rn^*/rn^* genotypes. The internal reflectance was higher in loin of the $RN^-/-$ genotypes compared with the

rn^+/rn^+ and rn^+/rn^*, rn^*/rn^* genotypes. The RN^- allele was dominant over the rn^+ and rn^* alleles in redness and yellowness, giving more red (high a^* value) and more yellow (high b^* value) colour. The rn^* allele tended to give lower redness (a^* value) and yellowness (b^* value) than the rn^+ allele. The RN^- allele was dominant over the rn^* allele in lightness (L^* value) giving lighter colour. The surface colour differences were mainly explained by differences in the distribution of myoglobin species. Finally, the L^* value was higher, whereas the pigment content, a^* value and the fraction of MbO_2 were lower in loin from entire males compared with females.

III. Significance of storage time on degree of blooming and colour stability of pork loin from different crossbreeds

The objective was to investigate the effect of ageing time (1 day vs. 8 days pm) and sire breed used in the crossbreed (Duroc-sired vs. Landraces-sired pigs) on blooming ability and colour stability of pork *M. longissimus dorsi* (LD). The colour was measured during blooming (0, 10, 30, 60, 90 min and 24 h after cutting) and during subsequent storage (1, 2, 3 and 6 days) at 3°C. The contents of deoxymyoglobin (Mb), oxymyoglobin (MbO_2) and metmyoglobin (MetMb) were calculated. Ageing improved the blooming of LD from both crossbreeds with increased content of MbO_2 and decreased content of Mb, resulting in increased lightness, redness and yellowness. Ageing had smaller effect on colour stability with slightly lower MetMb in aged meat. Crossbreed affected both blooming and colour stability. LD from Landrace-sired pigs bloomed more than LD from Duroc-sired pigs, but more MetMb was formed during subsequent storage, although at a low level in both crossbreeds. The present data show superior colour characteristics of fresh pork aged for 8 days.

IV. Significance of early *post mortem* temperature and pH decline on colour characteristics of pork loin

The significance of early *post mortem* (pm) temperature and pH decline and the level of the muscle metabolites creatine phosphate (CP) and adenosine triphosphate (ATP) on the colour of porcine *M. longissimus dorsi* was studied in a factorially designed experiment. Two stress levels *peri mortem* (minimal stress vs treadmill exercise and electrical stunning of the pigs) and four genotypes (Duroc boars crossed with Landrace-Yorkshire sows vs. Hampshire-Duroc boars crossed with Landrace-Yorkshire sows, including carriers and non-carriers of the halothane and RN^- genes, respectively) were included. Early pm muscle temperature and the accompanying pH decline had a significant influence on the pork colour independent of genotype. The combination of high temperature and low pH early pm increased lightness and yellowness, which is ascribed to inactivation of oxygen-consuming enzymes and protein denaturation. The effect of early pm temperature and pH on pork redness was more complex. It appears to be closely related to the extent of heat generation, CP and ATP levels and pH immediately pm in the muscle, which influences the activity of oxygen-consuming enzymes.

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V. Influence of dietary creatine monohydrate (CMH) and carcass cooling rate on colour characteristics of pork loin from different pure breeds

Increased creatine content in the muscle may delay *post mortem* (*pm*) lactate formation and postpone pH decline, hence potentially affect the colour of pork. The influence of dietary supplementation with 0 or 50 g creatine monohydrate (CMH)/d for 5 days prior to slaughter and two cooling rates of pig carcasses on the colour characteristics of pork loin from purebred Duroc and Landrace pigs was investigated. CMH increased the content of creatine phosphate in pork loin measured immediately following bleeding, delayed early *pm* pH decline and gave rise to less red and yellow colour, probably due to induction of a more pronounced oxidative muscle metabolism. A faster cooling rate *pm* induced darker and less yellow colour in loins from Duroc and Landrace pigs, while only loins from Landrace became less red. Loins from Duroc pigs were darker, less red and less yellow than loins from Landrace pigs, due to slower pH decline and a higher ultimate pH in these loins. Colour stability of pork loin during chill storage, measured as oxidation to metmyoglobin, was not affected by dietary CMH, cooling rate or pig breed. However, differences in colour during storage were related to differences in the degree of blooming, why more attention should be given to factors of importance for the degree of blooming in future studies of the colour of meat.

VI. Impact of *RN* genotype and storage time on colour characteristics of the pork muscles *longissimus dorsi* and *semimembranosus*

The effect of *RN* genotype on pH decline, ultimate pH, pigment content, blooming and colour stability during 6 days of storage at 5°C was studied in two pig muscles, *M. longissimus dorsi* (LD) and *M. semimembranosus* (SM), and furthermore the effect of anaerobic storage time (2-day- vs. 9-day-ageing) on the same parameters was examined. The *post mortem* pH decline was faster and the ultimate pH lower in LD and SM of the RN^- genotype compared with corresponding muscles from the rn^+ genotype. Pork of the RN^- genotype was initially lighter and more red and yellow than pork of the rn^+ genotype due to higher degree blooming, which might be explained by the faster pH decline and/or lower ultimate pH. The level of MbO_2 was decisive for the redness of both muscles during storage in air despite a higher presence of MetMb. Pork of the RN^- genotype was thus redder than that of the rn^+ genotype throughout storage in air despite higher oxidation to MetMb. Ageing for 9 days in chill improved the blooming potential in pork of both genotypes compared with 2-day-ageing, resulting in superior meat colour. However, only in pork from the RN^- genotype, the colour was not negatively affected by ageing time upon storage in air.

General discussion

Contribution of pigment content, myoglobin species and internal reflectance to colour

Colour is a sensation of light incident to the eye that arises from the interaction between light and matter. The colour of meat arises from a reflectance spectrum as a result of selective light absorbance by heme proteins, mainly myoglobin, imposed upon a spectrum formed by light scattered from the meat structure (Swatland, 1994).

It is well known that pigment content influences meat colour with higher pigment content giving the meat a darker red colour, e.g. beef, than lower pigment content, which gives a lighter and more pink colour, e.g. pork. The effect of the rate and extent of pH decline on pork paleness is also well established, with PSE pork as the most extreme (Bendall & Swatland, 1988). The lightness/paleness of pork is related to light scattering both due to the myofibrillar structure of meat (Offer *et al*., 1989; Swatland, 1992, 1995, 2002) and to the degree of protein denaturation (Swatland, 1992). The three myoglobin species found in fresh meat, Mb, MbO_2 and MetMb, have different reflectance spectra and hence different colours, and tristimulus colour parameters (L*, a* and b* values) are shown to vary according to the myoglobin species (Johansson, 1989; Fernández-López *et al*., 2000; Lindahl, Lundström & Tornberg*,* 2001, Paper I). The relative contents of the individual myoglobin species in the meat surface depend on the rates of autoxidation, MetMb-reduction and oxygen-consumption (Renerre, 1990). All these reactions are influenced by pH, which accordingly influence meat colour. Meat colour is also affected by the rate and extent of pH decline via protein denaturation.

Single correlations between pigment content or internal reflectance (FOP) and different colour parameters (Lundström *et al*, 1988; Feldhusen, 1994a; Tam *et al*, 1998; Newcom *et al*., 2004), as well as correlations between pH and colour (Tam *et al*, 1998; Hambrecht, Eissen & Verstegen, 2003; Moeller *et al*., 2003; Newcom *et al*., 2004) are found in the literature. However, these correlations explain only part of the variation, indicating that also other factors are important for the perceived meat colour. The aim of the first study (Paper I) was to evaluate the contribution of pigment content, myoglobin species and internal reflectance to the colour of pork using multivariate analysis. It was found that the combination of pigment content, myoglobin species and internal reflectance (FOP) could explain 86 to 90% of the variation in colour of normal pork. The myoglobin species were as important as the pigment content for all the tristimulus colour parameters. Unexpectedly, the internal reflectance (FOP) was only important for the b* value and not for the L* value. The reason for this was probably that only pork of normal meat quality with low extent of protein denaturation and not PSE pork was included.

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The low influence of the relative content of MbO_2 and the high influence of MetMb on the a* value (Paper I) were also unexpected, since the PLS predictions were performed on data from colour measurements on freshly cut pork directly after blooming. This might be explained by the fact that relative ratios of the myoglobin species (summing up to 1) and standardised variables were used in the PLS prediction, which increased the importance of the low, but variable content of MetMb. When interpreting the results, it should therefore be understood that the myoglobin species are important for colour with the positive and negative regressions shown, but that the relative magnitude of each species might not be proportional with the used procedure with standardised variables. See further discussion below.

Effects of genotype and peri-mortal treatments on colour

The colour of pork was influenced by genotype (Papers I, II, III, IV, V & VI), dietary supplementation with CMH (Paper V), pre-slaughter stress (Paper IV) and carcass cooling rate (Paper V). The variations in colour were all related to differences in early *post mortem* temperature and pH decline and/or ultimate pH, which affected the relative content Mb and MbO_2, i.e. blooming, and to some extent the relative content of MetMb. Furthermore, time of ageing also influenced colour by changing the ability to bloom (Papers III & VI).

A faster pH decline early *post mortem* (Fig. 4-5; Papers II, IV, V & VI) resulted, without any exception, in redder colour, with more or less marked differences (Fig. 6-7; Papers II, IV, V & VI) and often in lighter (Papers II, IV & V) and more yellow (Papers II, IV, V & VI) colour. The redder colour was related to higher relative content of MbO_2 (Papers II, V & VI). The effect was independent of whether the pH decline was a result of genotype (Papers II, V & VI), dietary supplementation with CMH (Paper V), pre-slaughter stress (Paper IV) or carcass cooling rate (Paper V). The faster pH decline was accompanied by differences in ultimate pH in some of the studies (Papers II, IV, V & VI). However, loins from Landrace-sired pigs were slightly redder compared with loins from Duroc-sired pigs, and the redness was related to a higher relative content of MbO_2, despite no difference in pH decline or ultimate pH was found (Paper III).

The faster pH decline in Landrace LD compared with Duroc LD in Paper V has to be ascribed to differences in muscle metabolism as no difference in temperature between the breeds was found. Pure pig breeds were used in this study (Paper V), whereas crossbreeds, Landrace and Duroc sires with Landrace×Yorkshire×Duroc dams, were used in the study in Paper III. The genetic variation between Landrace and Duroc was consequently much lower in the latter study, which might be the reason for the observed equal pH declines and ultimate pH. Anyhow, colour differences between the crossbreeds were found, and the only difference in the studied variables was a significantly slightly lower temperature at bleeding and 15 min *post mortem* in LD of Landrace-sired compared with Duroc-sired pigs.

The colour differences between the *RN* genotypes in Hampshire crossbreeds were related to both differences in pH decline and in ultimate pH (Papers II & VI). The differences in colour between LD and BF from Hampshire pigs compared

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with those from Landrace and Yorkshire pigs in the first study (Paper I) were probably also an effect of the RN^- allele. Differences in ultimate pH were observed in that study, but no measurements of the pH decline were performed. LD and BF from Hampshire (Paper I) were redder and more yellow than muscles from Landrace and Yorkshire as were LD (Paper II & VI) and SM (Paper VI) from carriers of the RN^- allele compared with those from non-carriers. The colour differences were related to higher relative contents of MbO_2 and MetMb and lower relative content of Mb, and furthermore to a higher internal reflectance (FOP). Increased relative content of MetMb was also observed during storage in carriers of the RN^- allele compared with non-carriers in the study in Paper VI. The higher relative content of MetMb was in all these three studies (Papers I, II & VI) at the expense of a lower relative content of Mb, indicating a low oxygen consumption rate in muscles of the RN^- genotype. However, this disagrees with the more oxidative metabolism reported in carriers of the RN^- allele (Lebret *et al.*, 1999). The present studies (Papers I, II & VI) show that a low level of Mb is more important for the perceived redness than only a high level of MbO_2, and that a low level of Mb can be achieved by a slightly higher level of MetMb. This fact explains the result of the PLS prediction of the a* value in paper I, where the relative contents of Mb and MetMb were significant variables for redness, whereas the relative content of MbO_2 was not.

The ratio of the different myoglobin species observed at the meat surface is dependent on the thickness of the different myoglobin layers as well as on the depth of light penetration during the colour measurements. The observed differences in the relative myoglobin fractions (Papers I, II, VI) could accordingly either depend on different thicknesses of the different myoglobin layers or on differences in light penetration. The meat surface may be more or less translucent depending on the rate of *post mortem* pH decline, ultimate pH and the extent of protein denaturation (Bendall & Swatland, 1988; Feldhusen, 1994a). The penetration depth of light decreases as an effect of increased light scattering due to an increased amount of water outside the myofibrillar space induced by the pH decline (Feldhusen, 1994a). At lower pH, a thicker layer of MbO_2 is formed (Ledward, 1992), and the formation of MetMb increases (Echevarne, Renerre & Labas, 1990; Tam *et al.*, 1998; Zhu & Brewer, 1998a), while at a higher pH, a thinner layer of MbO_2 and less MetMb are formed. Considering the higher internal reflectance in pork from Hampshire (Paper I) and carriers of the RN^- allele (Paper II), the most likely explanation for the high redness is decreased light penetration, so that the Mb layer is not reached. This is also supported by the more oxidative metabolism in carriers of the RN^- allele (Lebret *et al.*, 1999), which would have increased the relative content of Mb.

Pigs from different breeding lines and different crossbreeds were used in the studies of the *RN* genotypes, which might explain differences in the results. No difference in pH decline between the *RN* genotypes was found in the study in Paper IV, however, a lower ultimate pH was observed in LD from carriers of the RN^- allele compared with non-carriers (Fig. 4). This resulted in significantly higher lightness, but numerically only slightly higher redness (Fig. 6) in the latter, which indicates differences in light scattering properties, but not in the myoglobin species, which were not estimated in that study.
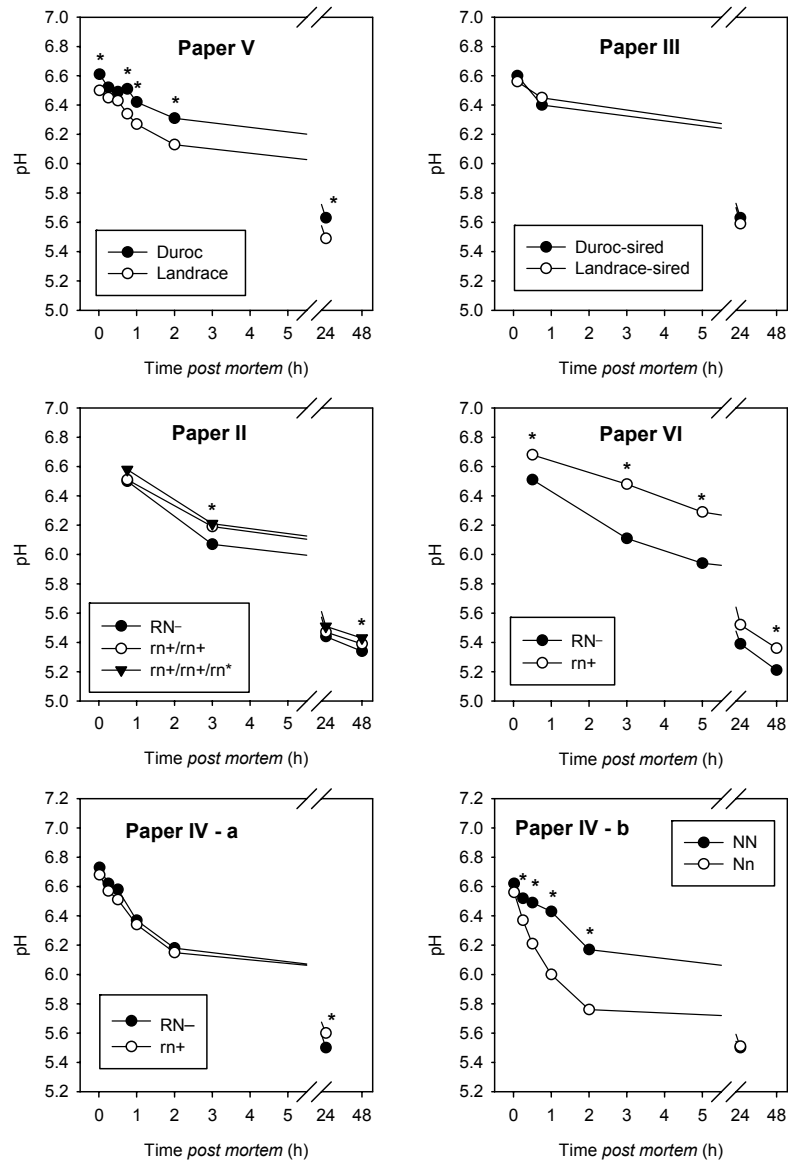
48

Fig. 4. pH decline and ultimate pH in LD from different pig genotypes. Duroc and Landrace (Paper V); Duroc-sired and Landrace-sired (Paper III); *RN* genotypes: *RN⁻/−, rn⁺/rn⁺, rn⁺/rn⁺/rn** (=group including *rn⁺/rn** and *rn*/rn** genotypes; Paper II); *RN* genotypes: *RN⁻* (including *RN⁻/RN⁻, RN⁻/rn⁺, RN⁻/rn**) and *rn+* (including *rn⁺/rn⁺, rn⁺/rn** and *rn*/rn** genotypes; Paper VI); *RN* genotypes: carriers (*RN⁻*) and non carriers (*rn+*) of the *RN⁻* allele (Paper IV-a); Halothane genotypes: non-carriers (*NN*) and heterozygous carriers (*Nn*) of the halothane allele (Paper IV-b). Significant differences in pH between genotypes are indicated by *, (P< 0.05).
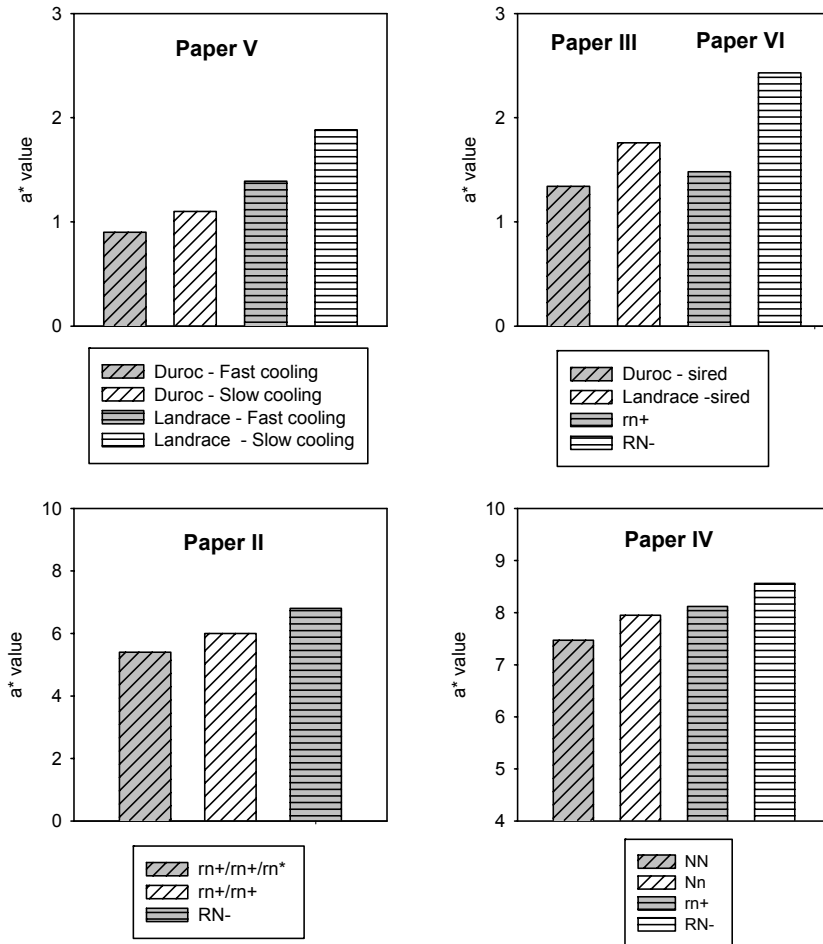
Fig. 5. pH decline and ultimate pH in LD from pigs with different treatments: cooling rate (Paper V-a), dietary supplementation with CMH (Paper V-b), pre-slaughter stress in *RN* genotypes; carriers (*RN⁻*) and non-carriers (*rn+*) of the *RN⁻* allele (Paper IV-a) and Halothane genotypes: non-carriers (*NN*) and heterozygous carriers (*Nn*) of the halothane allele (Paper IV-b). Significant differences between treatments are indicated by *, (P< 0.05).

Fig. 6. Redness (a* value) in LD from different pig genotypes. Duroc and Landrace, including fast and slow cooling rates (Paper V); Duroc-sired and Landrace-sired (Paper III); *RN* genotypes: $RN^-/-$, rn^+/rn^+, $rn^+/rn^+/rn*$ (=group including $rn^+/rn*$ and $rn*/rn*$ genotypes; Paper II); *RN* genotypes: RN^- (including RN^-/RN^-, RN^-/rn^+, $RN^-/rn*$) and $rn+$ (including rn^+/rn^+, $rn^+/rn*$ and $rn*/rn*$ genotypes; Paper VI); *RN* genotypes: carriers (RN^-) and non carriers ($rn+$) of the RN^- allele and halothane genotypes: Non-carriers (*NN*) and heterozygous carriers (*Nn*) of the halothane allele (Paper IV).
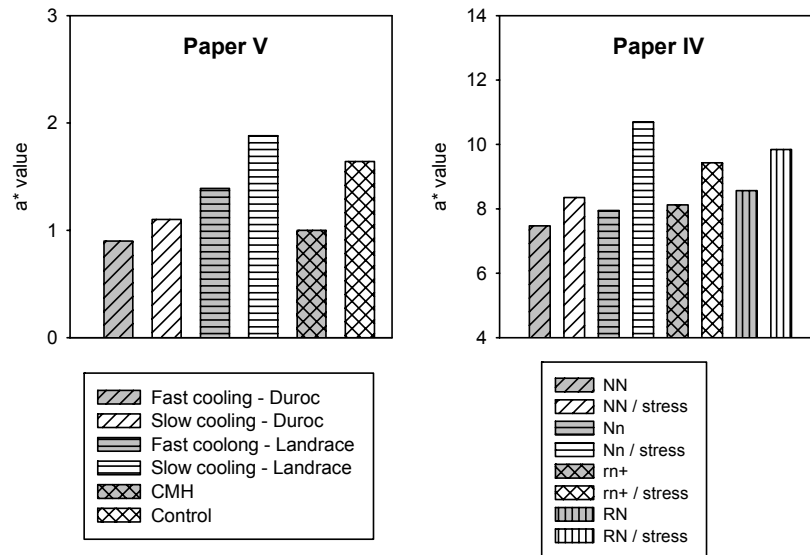
Fig.7. Redness (a* value) in LD from pigs with different treatments: cooling rate (Paper V), dietary supplementation with CMH (Paper V) and pre-slaughter stress in *RN* genotypes; carriers (*RN⁻*) and non-carriers (*rn+*) of the *RN⁻* allele (Paper IV) and halothane genotypes: non-carriers (*NN*) and heterozygous carriers (*Nn*) of the halothane allele (Paper IV).

Relationship between temperature/pH and colour

The results in Papers IV & V were used to investigate the influence of early *post mortem* temperature and pH progress on the L*, a* and b* values using PLS prediction. There was a large variation in the rate of pH decline, however, small variation in ultimate pH in the study presented in Paper IV (Fig. 5). Moreover, the pre-slaughter treatment induced a variation in muscle temperature early *post mortem* between 39.3°C and 41.6°C at 30 min *post mortem* in that study. The variation in rate of pH decline was smaller in the study presented in Paper V, making the variation in ultimate pH larger in relation to the variation in pH decline (Fig. 4-5). No significant variation in muscle temperature was found during the first hour *post mortem* in that study, but different cooling rates induced a variation in muscle temperatures with between 29°C and 32°C at 1.5 hours *post mortem*.

The studies showed that early *post mortem* muscle temperature (Papers IV & V), subsequent cooling rates (Paper V), the accompanied pH decline (Papers IV & V) and ultimate pH (Paper V) had a significant influence on pork colour independent of genotype. The effect of early *post mortem* temperature and pH on the redness of pork was far from straight forward (Fig. 7-8). A higher redness on the meat surface was obtained when pH early *post mortem* declined below approximately 6.0, while the muscle still generated heat to a temperature above approximately

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38°C and hereby compensated for the simultaneous cooling (Paper IV). This effect can be ascribed to inactivation of oxygen-consuming enzymes, which promote the oxygenation of myoglobin, i.e. blooming. In contrast, the degree of redness of the meat surface decreased if *post mortem* pH was higher than approximately 6.0, when the muscle temperature had started to decrease below approximately 38°C, as this rendered high enzymatic oxygen consumption the day after slaughter and diminished the degree of oxygenation of myoglobin (Papers IV & V).

Furthermore, the redness of pork appeared to be closely related to the extent of heat generation, CP and ATP levels and pH immediately *post mortem* in the muscle (Papers IV & V). Dietary supplementation with CMH in the days prior to slaughter increased the content of CP in the pork muscle and delayed early *post mortem* pH decline (Paper V). CMH supplementation gave rise to less red and yellow colour in the pork, which most probably can be explained by induction of a more pronounced oxidative muscle metabolism immediately *post mortem*. Data in Paper IV suggest that the oxygen-consuming activity is more preserved, leading to lower redness and yellowness, if glycolysis to lactic acid is delayed by utilising CP and ATP during heat generation immediately *post mortem*.

Two different temperature effects on lightness and yellowness were found, depending on whether the temperature was related to internal muscle temperature and metabolic activity early *post mortem* or to external cooling (Papers IV & V). The combination of high temperature and high pH early *post mortem* prior to cooling, induced darker and less yellow colour (Papers IV & V), as did also high ultimate pH (Paper V). This effect can be ascribed to it that the activity of oxygen-consuming enzymes was probably more preserved when high temperatures, accompanied by high pH values, prevailed early *post mortem*. At a later stage *post mortem*, high temperature, accompanied by low pH as an effect of slow cooling, induced lighter and more yellow colour due to more pronounced protein denaturation and to higher inactivation of oxygen-consuming enzymes.
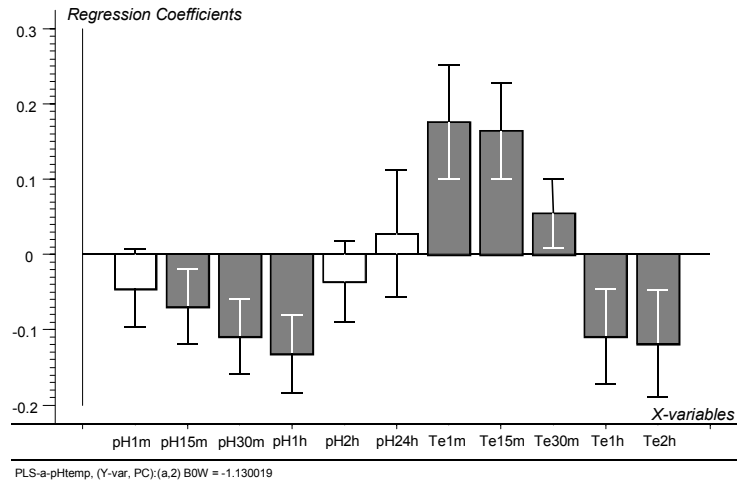
PLS-a-pHtemp, (Y-var, PC):(a,2) B0W = -1.130019

Figure 8. Regression coefficients from PLS predictions of the a* value in *M. longissimus dorsi* from different pig genotypes (DDLY$_{NN}$, DDLY$_{Nn}$, HDLY$_{rn+}$, HDLY$_{RN–}$) subjected to different treatments (Control and ExEl). X-variables: pH and temperature (Te) 1 min, 15 min, 30 min, 1 hour, 2 hours and pH 24 hours, N=180. Significant regression coefficients, due to Martens' uncertainty test, are shown in gray. From Paper IV.
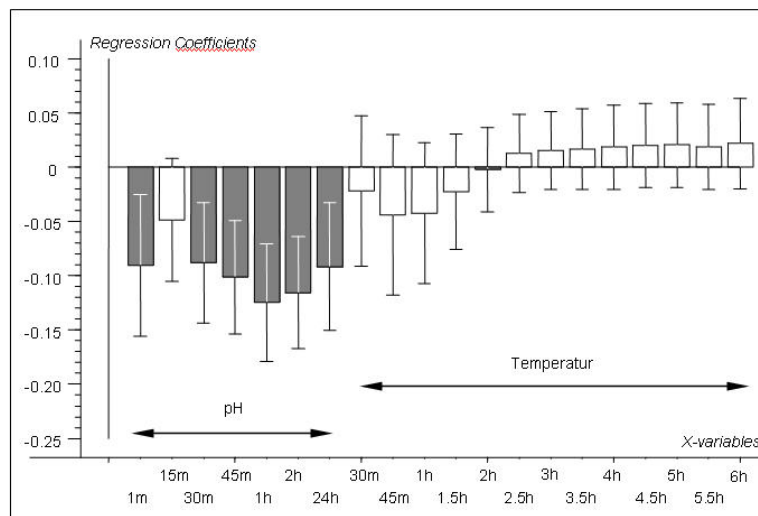

Fig 9.Regression coefficients from PLS predictions of the a* value in pork loin. X-variables: pH 1 min, 15 min, 30 min, 45 min, 1 h, 2 h and 24 h and temperature (Te) 1, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330 and 360 min. Significant regression coefficients, due to Martens' uncertainty test, are shown in gray. From Paper V.

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Aerobic storage

Immediately when meat is exposed to air, Mb reacts with oxygen to form MbO_2, and the meat blooms to a cherry-red or pink colour. Upon further storage myoglobin oxidises to MetMb, and the meat discolours. Colour changes during blooming (Paper III) and during chill storage for 6 days in air were studied (Papers III, V & VI).

As reflected in redness, yellowness and relative content of MbO_2, blooming was most rapid during the first 30 min of exposure to air, but continued for 24 hours (Paper III). This is in contrast to a previous study, which reported that only 10 min was required for the a* and b* values to stabilise during blooming (Brewer *et al*., 2001), however, it is more in line with the study of Zhu, Bidner & Brewer (2001), who concluded that blooming is not completed within 30 min. A continuous increase in lightness, redness and yellowness during blooming on the first day of storage has been reported previously (Juncher *et al*., 2001), whereas only increase in lightness and yellowness was reported by Hansen *et al*. (2004) and Rosenvold & Andersen (2003a).

The level of blooming was affected by genotype with higher degree of blooming in LD from Landrace compared with Duroc pure breed (Paper V) or crossbreed (Paper III) pigs and in LD and SM from non-carriers of the RN^- allele compared with carriers (Paper VI). These differences are related to the differences in the rate of pH decline and ultimate pH as discussed above.

Redness decreased during an additional five days of storage (Papers III & VI), which is in line with previous studies (Juncher *et al*., 2001; Rosenvold & Andersen, 2003a; Hansen *et al*., 2004). The decreased redness was a result of oxidation of myoglobin, clearly reflected in increasing levels of MetMb and simultaneous decrease in MbO_2 levels. The oxidation to MetMb was slightly higher in LD from Landrace-sired compared with Duroc-sired pigs, although the MetMb levels were low in both and had no dramatic effect on colour (Paper III). The level of MbO_2 was decisive for the redness of both LD and SM during storage in air, even though higher levels of MetMb was present in muscles from the RN^- genotype (Paper VI).

The progress in colour changes during storage of LD from pure breed Duroc and Landrace pigs (Paper V) was neither influenced by breed nor by treatments dietary with CMH supplementation or cooling rate. The colour changes during storage could solely be explained by oxidation of MbO_2 to MetMb, while the colour differences due to the effect of CMH, cooling rate and breed could be explained by differences in MbO_2, i.e. by the degree of blooming. In line with the present study, Milligan *et al*. (1998) found no effect of cooling rate on lightness, redness and yellowness of pork loin during 3 days of retail case display at 2°C.

Ageing

Independent of genotype, ageing for 8-9 days initially increased lightness, redness and yellowness in pork muscles compared with ageing for only 1-2 days due to increased blooming with a higher level of MbO_2 and a lower level of Mb (Papers

III & VI). This is in agreement with a previous study by Zhu *et al.* (2001) who also found that ageing increases the ability of vacuum-packaged pork to bloom. This is caused by reduced oxygen consumption potential as a function of time *pm* hereby allowing more rapid blooming (Atkinson & Follet, 1973). In agreement with the present studies, Apple *et al.* (2001) reported increased lightness, redness and yellowness after 4 and 8 weeks of vacuum storage of pork, whereas Apple *et al.* (2002) found increased lightness, decreased yellowness, but no effect on redness after 3 weeks of vacuum storage. Moreover, Rosenvold & Andersen (2003a) reported higher redness and yellowness, however, no difference in lightness after 8 days of vacuum storage. Frederick *et al.* (2004) found numerically lower lightness and higher redness and yellowness in pork of LD after 25 or 50 days of vacuum storage compared with LD 24 hours *pm*. Schluter *et al.* (1994) reported no difference in lightness and a lower redness and yellowness in vacuum-stored LD after 10 days compared with 7 days, but only lower yellowness after 14 days of storage.

Upon further storage in air, pork from Duroc-sired and Landrace-sired pigs (Paper III) only showed a minor effect of ageing on myoglobin oxidation, with slightly lower MetMb in aged meat after 6 days of storage. This is in contrast to the study of Rosenvold & Andersen (2003a), where the higher redness in the aged LD decreased faster during a subsequent 5-day retail display to the same levels as in not aged LD, indicating a more rapid colour-fading in aged pork, which is in agreement with data on colour stability of aged beef (O'Keefe & Hood, 1980-81).

The rate of myoglobin oxidation after ageing was more pronounced in pork from the rn^+ genotype than from the RN^- genotype, as was also reflected in a more rapid discoloration measured as decrease in redness (Paper VI). The better colour stability of aged pork from the RN^- genotype compared with that from the rn^+ genotype might be explained by a higher MetMb-reducing potential in these muscles, as expected from their more oxidative nature (Lebret *et al.*, 1999). A high level of NADH enhances MetMb reduction, as both enzymatic and non-enzymatic MetMb-reducing activity are shown in pork (Mikkelsen, Juncher & Skibsted, 1999).

However, during prolonged storage (> 4 days), a marked increase in myoglobin oxidation in aged LD and SM of the rn^+ genotype was observed, coinciding with an increase in Mb and a higher decrease in MbO_2 (Paper VI). This was reflected in decreased lightness in both muscles and decreased yellowness in SM. The increased oxidation to MetMb was probably an effect of the higher level of Mb, since Mb oxidises faster than MbO_2 (Wazawa, *et al.*, 1992; Shikama, 1998). These reactions were seen in both muscles after ageing, and the reactions were most pronounced in muscles of the rn^+ genotype. The reason for the more reductive condition that leads to deoxygenation of MbO_2 to Mb is not known.

Colour measurements

The relative content of the different myoglobin species, Mb, MbO_2 and MetMb, was estimated with two different methods, the Krzywicki (1979) method (Papers I & II) and the method using K/S values (Papers III, V & VI). The Krzywicki

(1979) method was applied in the first studies, when a Hunterlab Colour Quest instrument was used (Papers I & II). That method was not applicable in the later studies, where a Minolta CM-2600d spectrophotometer was used, since negative values for the calculated relative content of MetMb were obtained. The Hunterlab Colour Quest instrument is a stationary laboratory instrument with a large integrated sphere (152 mm diameter) and 25 mm measuring aperture, whereas the Minolta CM-2600d is a portable instrument with a small sphere (80 mm diameter) and 8 mm measuring aperture. The shape of the reflectance curves was comparable, but the level of the reflectance values and the L*, a* and b* values was much lower with the Minolta CM-2600d instrument compared with the Hunterlab Colour Quest instrument. These instrumental differences might be the reason for the failures in calculation of the relative contents of the myoglobin species using the Minolta CM-2600d instrument. The adjustment for attenuation of light by pigment-free meat at 730 nm (Krzywicki, 1979) might be the problem. Using 8 mm aperture compared with 25 mm aperture on the Hunterlab Color Quest instrument resulted in a flatter reflectance curve, especially above 600 nm (Lindahl, unpublished data). Another reason might be that the wavelengths used by Krzywicki (1979) are not optimal for the calculations, which was indicated by Tang, Faustman & Hoagland (2004) for absorbance measurements. There is a need for further development of methods for estimation of the myoglobin species at the meat surface. Nevertheless, the results obtained with both of the used methods were comparable.

Conclusions

- Pigment content, myoglobin species and internal reflectance influence the colour of fresh pork. The colour hue, as measured in L*, a* and b* values, is highly affected by the relative content of the myoglobin species.

- The early progress in *post mortem* temperature and pH decline affects the colour of pork independent of whether this is triggered by variations in genotype, feed-induced changes, pre-slaughter stress or cooling rate.

- The level of MbO_2 is more important for the colour of pork during retail display, than the level of MetMb. A high level of MbO_2 preserves high redness despite of oxidation to MetMb.

- Genetics giving rise to variation in the colour of pork is related to differences in pigment content and in muscle metabolism. The latter influences the redox status of the muscle and the activity of oxygen-consuming and MetMb-reducing enzymes and accordingly the relative proportion of the myoglobin species:

  - The colour of pork from Landrace pigs is generally lighter, redder and more yellow than pork from Duroc pigs due to higher level of MbO_2 at the meat surface, i.e. more intense blooming.

  - The colour of pork from Hampshire pigs is highly related to the *RN* genotype. Pork from carriers of the *RN⁻* allele is generally lighter, redder and more yellow than that from non-carriers of the *RN⁻* allele, and the *rn**(V199I) allele tends to decrease redness and yellowness.

- Ageing of pork for a week increases the ability of pork to stimulate oxygenation of Mb to MbO_2, i.e. blooming, without compromising the colour stability during four days of subsequent retail display. Hence, ageing results in lighter, redder and more yellow pork colour.

Future perspectives

The studies in this thesis have shown the importance of *peri mortal* events and ageing on the colour characteristics of fresh pork. Furthermore, it was shown that the genetic variation in pork colour is related to muscle metabolism. Future studies should focus on the following questions:

- Considering the increasing market of meat packaged in modified atmospheres (MAP) with high oxygen content, more fundamental understanding of the influence of high oxygen partial pressure on blooming and colour stability of pork is needed. Especially, packaging of aged pork in MAP has to be exploited, as the longer period of retail display might compromise both the superior colour found in aged pork in the present study and its colour stability.

- In view of the importance of blooming for the redness of pork, further studies should focus on factors that influence the ability of pork to bloom. Improved understanding of variations in muscle metabolism related to the redness of pork would increase the potential in breeding for optimal pork colour. Modern pig breeding has resulted in decreased pigment content, making it even more critical to optimise the redness of pork.

- Future studies should also focus on a more fundamental understanding of *peri-mortal* events on muscle metabolism and their mutual effects on meat quality attributes, especially in view of using strategic tools, e.g. feeding, to control muscle metabolism and hereby optimise mutual dependence meat quality attributes, e.g. the colour and water-holding capacity of pork.

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