Relationship between beef colour and myoglobin

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Preface

This thesis is part of my Master in Food Technology at Wageningen UR. This subject was carried out at the Quality in Chains department of Agrotechnology & Food Innovations (A&F) in co-operation with the Product Design and Quality Management Group of Wageningen UR. I worked on this thesis from January till August 2005 at A&F. During my study I became interested in Meat Science and the subject of this thesis fits perfectly to my interests. During my stay at A&F I had all freedom and responsibility to conduct my research. This made I really enjoyed working on this thesis. Moreover, it increased my knowledge of Meat Science very much. I would like to thank Ulphard Thoden van Velzen, my supervisor at A&F, and Jozef Linssen, my supervisor at the University, for their help during this study. I also want to thank Ronald Holtmaat from ProMessa (Deventer, NL) who kindly provided the meat for this study. Furthermore I would like to thank Aart Zegveld, Dianne Somhorst and Janny Slotboom who helped me with my practical work at A&F.

Abstract

The most important quality attribute of fresh beef is its colour. The colour of meat depends on three forms of myoglobin. The centre of the meat contains deoxymyoglobin and gives it its purple colour. The outside of the meat, which is in contact with oxygen, contains oxymyoglobin. This gives the meat its desirable red colour. In time, these two forms of myoglobin are transformed to metmyoglobin. Metmyoglobin is responsible for the undesirable brown colour. Factors involved in the discolouration of beef are autoxidation, metmyoglobin reductase activity and oxygen consumption rate. Discolouration is not caused by one factor, but by a combination of several technological, intrinsic and extrinsic factors.

The aim of this thesis is to investigate the possibility of a relationship between the instrumental colour measurements (RGB measurement) and myoglobin.

In this research rib eye was used which was cut out of one animal to reduce some technological, intrinsic and extrinsic factors. Meat samples were packaged aerobically and in modified atmosphere (MA) (60% O_2, 30% CO_2, 10% N_2) and stored in cold storage rooms at 2°C and 7°C. The colour of the meat was measured in R-, G- and B-values using a digital camera and a computer program. Determination and quantification of the three forms of myoglobin was done by using the method of Krzywicki.

It was not possible to relate beef colour to myoglobin using a digital camera and computer software.

Measuring the R-, G- and B-values, the surface method was found to be more accurate than the point measurement.

With the pigment analysis method of Krzywicki it was not possible to distinguish between deoxymyoglobin and oxymyoglobin. During the extraction deoxymyoglobin was converted to oxymyoglobin. Nevertheless the method was suitable for the quantification of oxymyoglobin and metmyoglobin.

During the experiment the R-value of the aerobic samples declined fastest. The R-value became less than at day 0 after 2 (7°C) and 6 (2°C) days. A lower storage temperature results in a longer colour stability of the meat. The MA packaged meat remained red for the duration of the experiment. In none of the experiments changed the G- or B-value considerably in time.

In all experiments the percentage of oxymyoglobin and metmyoglobin showed a tendency with the corresponding R-values, but the correlation coefficient was low. Against all expectations, no analytical relation could be made between the R-value and the percentage of oxymyoglobin. This was caused by the large biological variation in meat colour, the inaccuracy of the RGB measuring method and the method of Krzywicki.

All meat samples showed the relation that when the percentage of oxymyoglobin decreased, the percentage of metmyoglobin increased. Meat stored at 7°C showed an accelerated increase in the percentage of metmyoglobin and a decrease in the percentage of oxymyoglobin in relation to meat stored at 2°C.

R-, G- and B- values can be used as an indication to estimate the pigment composition of the meat. This is nothing more than a superficial relation.

Samenvatting

Het meest belangrijke kwaliteitsatribuut van vers rundvlees is de kleur. Kleur van het vlees hangt af van de drie verschillende vormen van myoglobine. De kern van het vlees bevat deoxymyoglobine en heeft een paarse kleur. De buitenkant van het vlees, dat in contact staat met zuurstof, bevat oxymyoglobine. Dit geeft het vlees de gewenste rode kleur. Na verloop van tijd worden deze twee vormen myoglobine omgezet in metmyoglobine. Metmyoglobine is verantwoordelijk voor de ongewenste bruine kleur van vlees.

Factoren die betrokken zijn bij de verkleuring van rundvlees zijn auto-oxidatie, metmyoglobine reductase activiteit en het zuurstof consumptie niveau. Verkleuring van vlees wordt niet veroorzaakt door één enkele factor, maar door een combinatie van verschillende technologische, intrinsieke, en extrinsieke factoren.

Het doel van dit onderzoek is om een relatie aan te tonen tussen de instrumentele kleur van rundvlees en myoglobine.

In dit onderzoek zijn riblappen gebruikt, afkomstig uit één dier om verschillende technologische, intrinsieke en extrinsieke factoren te elimineren. Het vlees werd verpakt in omgevingslucht en in veranderde gassamenstelling (60% O_2, 30% CO_2, 10% N_2) en opgeslagen in koelcellen van 2°C en 7°C. De kleur van het vlees werd gemeten als rood, groen en blauw (RGB) waarde met behulp van een digitale camera en computerprogramma met de naam Peer V11. Het isoleren en kwantificeren van de drie vormen van myoglobine werd gedaan met behulp van de methode van Krzywicki.

Het was niet mogelijk om de kleur van rundvlees te relateren aan myoglobine met behulp van een digitale camera en computersoftware.

Voor het meten van de RGB waarde bleek de oppervlaktemethode nauwkeuriger dan de puntmethode.

De methode van Krzywicki bleek niet in staat om deoxymyoglobine en oxymyoglobine apart te detecteren. Tijdens de extractie werd deoxymyoglobine meteen omgezet in oxymyoglobine. Desondanks was de methode geschikt voor de kwantificatie van oxymyoglobine en metmyoglobine.

Bij de in omgevingslucht verpakte vleesmonsters daalde de R-waarde het snelst. De R-waarde daalde onder het niveau van dag nul na 2 (7°C) en 6 (2°C) dagen. Opslag bij een lagere temperatuur zorgt voor een langere kleur stabiliteit. Het vlees verpakt onder veranderde gassamenstelling bleef tijdens het hele experiment rood van kleur. De G- en B-waarde veranderde niet noemenswaardig veel gedurende het experiment.

In alle experimenten vertoont het percentage oxymyoglobine en metmyoglobine een duidelijke tendens met de gemeten R-waarde, maar de correlatie coëfficient is laag. In tegenstelling tot alle verwachtingen kon er geen analytisch verband worden aangetoond tussen de gemeten R-waarde en het percentage oxymyoglobine. Dit werd veroorzaakt door de grote biologische variatie in de kleur van het vlees en de onnauwkeurigheid van de RGB meetmethode en de methode van Krzywicki.

Bij alle vleesmonsters steeg het percentage metmyoglobine als het percentage oxymyoglobine daalde. Vlees dat bij 7°C is opgeslagen vertoonde een versnelde toename van het percentage metmyoglobine en afname in het percentage oxymyoglobine ten opzichte van vlees opgeslagen bij 2°C.
Rood, groen en blauw waarden van vlees kunnen worden gebruikt als een indicatie voor het voorspellen van de pigment samenstelling in het vlees. Dit is niet meer dan een oppervlakkige relatie.

Content

1 Introduction

The most important factor on which consumers base their judgement of a piece of meat is appearance. Appearance determines how consumers perceive quality and significantly influence purchasing behaviour. Therefore it is commercially important to understand the mechanisms how appearance of the meat changes in time.

Appearance is based on the combination of several factors. One of those factors is the colour of meat. The colour of meat is caused by myoglobin. Myoglobin is a haem protein and in the meat it is present in three different forms. These are deoxymyoglobin, oxymyoglobin and metmyoglobin. Meat colour depends on the ratio in which these three forms of myoglobin are present. When a piece of meat is cut, the centre has a purplish like colour, because the majority of the myoglobin is present in the deoxymyoglobin form. Consumers prefer the meat to have a bright red colour, which is present when the majority of the myoglobin is present in the oxymyoglobin form. In time, the two forms of myoglobin mentioned before are transformed to metmyoglobin. This gives the meat its undesirable brown colour.
The colour and discolouration of the meat depends on different factors. No single factor is responsible for the colour and discolouration of the meat. Some of these factors are the history of the animal, muscle type, storage and display conditions. In order to research the impact of these factors on the colour of meat it is necessary to determine the amount, and in which form, the myoglobin is present. The method that is used until now relies on the extraction and quantification of the three forms of myoglobin out of the meat. This is a time consuming process. This study attempts to relate the instrumental colour to the amount of the related forms of myoglobin. In this way it is less time consuming to determine the amount and form of myoglobin.

1.1 Aim

A new technology of measuring colour is using a digital camera in combination with computer software. In this way, the colour of the meat can be defined as an R-, G- and B-value instead of for example L a b. The aim of this thesis is to investigate the possibility of a relationship between the instrumental colour measurements (RGB measurement) and myoglobin. The two objectives are to optimise the instrumental colour measurement and to find an accurate method to quantify the different forms of myoglobin in the meat.

2 Literature review

Consumer's preferences for meat, from a sensory standpoint are influenced by appearance, tenderness, flavour and juiciness. The most important of these is appearance. Appearance determines how consumers perceive quality and significantly influences purchasing behaviour (Resurreccion, 2003).
The typical red colour of fresh meat can change during time to a less desirable brown colour. This is caused by the oxidation of the myoglobin, which gives the meat its red colour.

2.1 Myoglobin

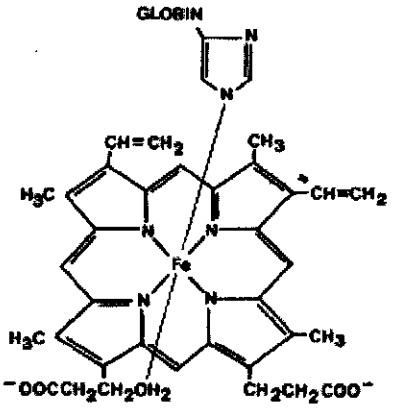


Figure 2.1: Structural representation of myoglobin (Hutchings, 1994).

Myoglobin is the haem protein primarily responsible for meat colour. In the living cell, it serves both as an oxygen storage and oxygen delivery function. The exact length of the polypeptide chain of myoglobin is species dependent, but contains approximately 153 amino acids in mammals and a secondary structure which is 80% alpha helix (Faustman and Cassens, 1990). Myoglobin is a monomeric, globular haem protein with a molecular weight of approximately 17.000 D. It possesses a haem prosthetic group, which is responsible for oxygen binding and confers an intense red or brown colour on the protein (Renerre, 1990). The haem prosthetic of myoglobin is composed of an iron atom, which is bound within a protoporphyrin ring by four of the iron atom's six co-ordination sites. The haem group is attached to the apoprotein at the fifth co-ordination site by a bond between the iron atom and a histidine residue. The sixth site is available for binding a variety of ligands (Faustman and Cassens, 1990).
The globin portion of the molecule serves to confer water solubility upon the hydrophobic haem group and protects the haem iron from oxidation (Cornforth and Jayasingh, 2004).
The haem iron may exist in a reduced ferrous (Fe^{+2}), or oxidized ferric (Fe^{+3}) form. Ferrous haem iron, that contains water at the sixth site is, called deoxymyoglobin. A piece of meat in which myoglobin is the predominant pigment form will appear purplish-red in colour. When oxygen occupies the sixth site of the ferrous haem, oxymyoglobin is formed. Oxymyoglobin is

responsible for the desirable cherry-red colour of fresh meat. These two reduced forms of myoglobin can be oxidized to the undesirable brownish red metmyoglobin, in which the haem iron is converted to the ferric state and water occupies the sixth site. Metmyoglobin is incapable of binding oxygen and is therefore physiologically inactive. Oxymyoglobin is more stable towards oxidation than deoxymyoglobin, due to hydrogen bonding between the bound oxygen and a distal histidine residue of the apoprotein. Metmyoglobin may be converted back to a physiologically active form by enzymes known as metmyoglobin reductases, but this is a difficult process (Faustman and Cassens, 1990).

The colour of meat and meat products depends on the concentration of meat pigments, the chemical state of these pigments and physical characteristics of the meat, e.g. light scattering properties. During storage, the rate of metmyoglobin accumulation on the surface of beef meat is related to many intrinsic factors (e.g. pH, muscle type, animal age, breed, sex etc.), extrinsic ones (pre-slaughter treatments, electrical stimulation, hot-boning, etc.) and technological ones (temperature, type of packaging, light etc.). In fresh meat, oxidation to metmyoglobin is affected by the reducing capacity of the muscle, oxygen availability and myoglobin autoxidation rate (Renerre, 1990).

Table 2.1: Colour perceived by consumer and consumer discrimination in relation to the percentage of metmyoglobin in the meat (Kropf, 2004).

% of metmyoglobin	Colour and consumer discrimination
0	Cherry-red, buy
20	Starting to see difference, still bought
40	Brown, not bought
60	Brown to grey-greenish, not bought

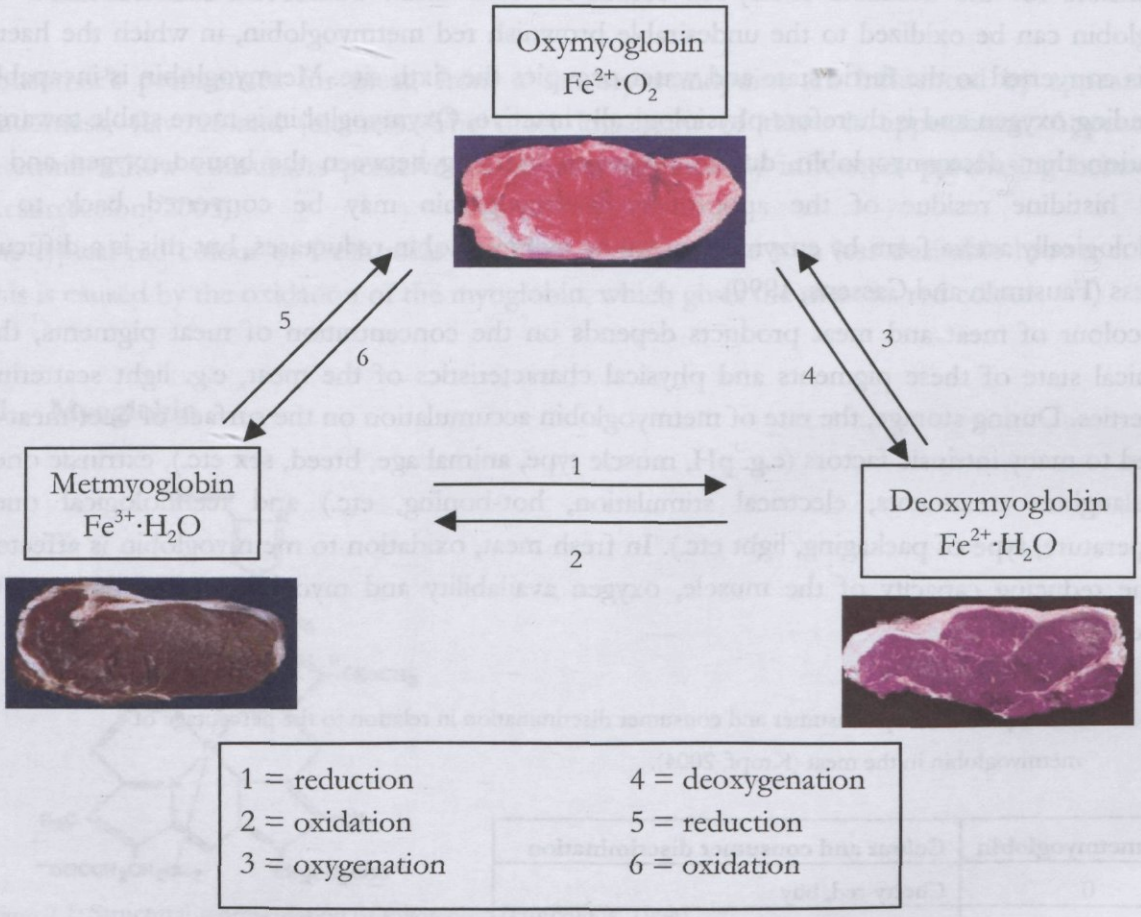


Figure 2.2: Three forms of myoglobin.

In figure 2.2 the three different forms of myoglobin are shown. The colour change between the three different forms of myoglobin can clearly be seen. In appendix 1 the reactions are written down that can take place during the oxidation of oxymyoglobin to metmyoglobin.
Immediately after cutting the meat is purplish-red (deoxymyoglobin). Deoxymyoglobin is very unstable and as oxygen from the air comes in contact with the exposed meat surfaces, it is absorbed and binds to the haem group. The meat surface blooms as the myoglobin becomes oxygenated and produces a cherry-red colour (oxymyoglobin). The meat will turn brown in time because of the formation of metmyoglobin. Metmyoglobin is very stable and is difficult to turn back into deoxymyoglobin or oxymyoglobin.

The three forms of myoglobin are all present in meat and are always in equilibrium with each other. On the surface of the meat, there is enough oxygen available and oxymyoglobin will be formed. But when oxygen penetrates in the meat it is used to oxidise reduced compounds. This results in an oxygen gradient ranging from saturation on the surface to zero in the centre of the meat. Between these zones there is a region with little oxygen. In this region metmyoglobin is formed.

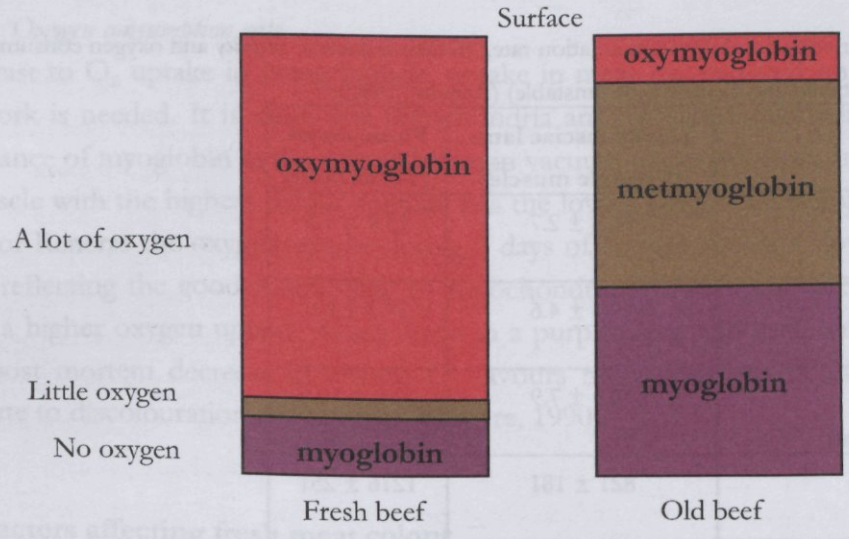


Figure 2.3: Generalised schematically description of the colour of the meat in relation to the oxygen availability.

2.2 Myoglobin oxidation and reduction mechanisms

According to several studies, the development of metmyoglobin depends essentially on the myoglobin oxidation rate, the enzymic ferrimyoglobin reduction and the oxygen consumption rate. The mechanisms involved and their relative importance in meat discolouration have been extensively studied but are not yet well established (Renerre, 1990).

2.2.1 Autoxidation

The most important reaction in the discolouration of the meat is the oxidation of the native ferrous form to metmyoglobin. This reaction is termed autoxidation since it proceeds with oxygen. The mechanism remains largely unknown, but involves a non-enzymic spontaneous oxidation by free oxygen, which determines the rate of discolouration. The reaction is highly temperature dependent with a Q_{10} of about 5 and an activation energy of 24 to 27.8 kcal mol^{-1}.
Other important factors are the pH of the meat, the presence of concentrated salts, oxidation-reduction mediators and catalytic amounts of heavy metals.
Biological factors such as muscle type can also play an important role. It was shown that less stable muscles have the highest autoxidation rate, as can be seen in table 2.2. Between beef muscles, variability in their vulnerability to haem pigment oxidation appears to be related to the amount of oxygen entering the exposed meat surface over time. This could depend on whether differences in myoglobin oxidation rates are determined by the pigment composition and its properties, and/or on the surrounding environment myoglobin (Renerre, 1990).

Table 2.2: Mean values for colour stability, autoxidation rate, MetMb reductase activity and oxygen consumption rate for Tensor fasciae latae (stable) and Psoas major (unstable) (Renerre, 1990).

	Tensor fasciae latae (Triangle muscle)	**Psoas major (Tenderloin)**
Colour stability ($\%R_{630} - R_{580}$, 30°C, 5 h)	16.3 ± 2.7	13.1 ± 1.8
Autoxidation rate (% MetMb h^{-1})	19.4 ± 4.6	29.2 ± 6.0
MetMb reductase activity (nmol MbO_2 min^{-1} g^{-1} meat)	34.9 ± 7.9	37.9 ± 10.6
Oxygen consumption rate (nmol O_2 min^{-1} g^{-1} meat)	821 ± 181	1216 ± 251

2.2.2 Metmyoglobin reductase activity

Fresh meat doesn't show the characteristic brown colour of metmyoglobin. Enzymes available in the meat reduce metmyoglobin (Fe^{3+}) into myoglobin (Fe^{2+}) which is able to bind oxygen and give the meat the desirable cherry-red colour. In time the amount of metmyoglobin in the meat rises and the meat turns brown. This is caused by a decrease in enzyme activity during ageing of the meat. The decrease in enzyme activity is caused by the decrease of substrate.

Possible enzymic pathways have been studied and it is now accepted that the reduction process in meat is primarily enzymic in nature with NADH as coenzyme (Watts *et al.*, 1966). In table 2.2 is shown the meat with the highest colour stability has the lowest metmyoglobin reductase activity.

Metmyoglobin reductase was found to be unaffected by the presence or absence of oxygen. *In situ* in meat the reduction of metmyoglobin is more complex than *in vitro* with the purified enzyme, because several substrates and intermediates are present. 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 and, ultimately, complete loss of structural integrity and functional properties of the mitochondria (research showed metmyoglobin reductase activity present in bovine muscles was localized in microsomes and mitochondria).

Other research has asserted that the activity of the reducing system is not the most important factor influencing the rate of metmyoglobin formation (Renerre, 1990).

2.2.3 Oxygen consumption rate

In contrast to O_2 uptake in living muscle, uptake in meat has received only little attention and more work is needed. It is clear that mitochondria and/or submitochondrial particles facilitate maintenance of myoglobin in the reduced state in vacuum packaged meat. In table 2.2 it is shown that muscle with the highest colour stability has the lowest oxygen consumption rate. According a study of Renerre the oxygen uptake during 8 days of storage shows a slow decrease in oxygen uptake, reflecting the good functioning of mitochondria post mortem. Meat with high ultimate pH has a higher oxygen uptake, which leads to a purplish red and dark colour. It is also shown that a post mortem decrease in muscle O_2 favours myoglobin autoxidation. All these factors contribute to discolouration of the meat (Renerre, 1990).

2.3 Factors affecting fresh meat colour

Many factors affect the colour and colour stability of meat and those of greatest importance are discussed in the following sections. The factors that influence the meat colour and stability are divided in intrinsic, extrinsic and technological factors. Meat is a complex food and no single factor is responsible for the discolouration of the meat, interaction between factors causes the discolouration of the meat.

2.3.1 Technological factors

- Temperature/ relative humidity

High temperatures are known to influence the colour stability negatively, to promote the bacterial growth and resulting in low oxygen tensions that facilitate autoxidation of myoglobin. High storage temperatures will move the brown metmyoglobin intermediate layer between the oxymyoglobin and deoxymyoglobin closer to the surface and metmyoglobin becomes visible with increasing time. The rate of discolouration with temperature is muscle dependent and at 10°C the rate is two- to five-fold higher than at 0°C. Optimal storage temperature for meat is therefore -1°C to –1.5°C, because the freezing point of meat is at -2°C.

High temperatures are also responsible for the loss of globin function of protecting the haem when there is deoxygenation of red oxymyoglobin and formation of deoxymyoglobin thus leading to an increased tendency for autoxidation.

Low temperatures promote the penetration of oxygen into the meat surface and oxygen solubility in tissue fluids. These maintain myoglobin in the oxymyoglobin form (Renerre, 1990).

When meat is properly packaged it should not discolour as a result of dehydration. The relative humidity does not decrease the colour stability of the meat but enhances other factors (Faustman and Cassens, 1990).

- Bacterial contamination

The specific role of bacteria in meat discolouration processes has received little research attention. The majority of the studies have focussed on the spoilage bacteria *Pseudomonas* spp. These bacteria are the dominant psychrotrophs found on aerobically packaged meat (Faustman and Cassens, 1990).

The moist atmosphere inside meat packages is ideal for bacterial growth. Growth of bacteria results in a drop in the oxygen tension at the surface of the meat resulting in an increase of metmyoglobin. Metmyoglobin is especially formed during the logarithmic growth of *Pseudomonas*, *Achromobacter* and *Flavobacterium*. Other bacteria can form by-products like hydrogen sulphide (H_2S) and hydrogen peroxide (H_2O_2). These by-products lead to a green discolouration of the meat (Renerre, 1990).

- Type of packaging

A meat package has three functions: the major function is to serve as protection from environmental variables. These include temperature, moisture or humidity, oxygen, airborne particles and light. The second function is to act as protection against biological contamination such as that from humans and micro-organisms. The last function is to serve as a marketing tool (Kropf, 2004).

There are three types of packaging possible for meat. These are over wrap with cling foil, vacuum and atmospheric packaging.

In the first type of packaging the meat stays red for a few days, depending on the oxygen permeability of the foil. During the first few days after packaging the meat is retained in the oxymyoglobin form due to the available oxygen inside the package. The foil gives minimal protection to bacterial contamination. The predominant spoilage bacteria in this type of packaging are the aerobic bacteria like the strictly aerobic *Pseudomonas*. After four to eight days after packaging the meat will be spoiled, also depending on the temperature of storage and the initial contamination level (Kropf, 2004).

The distribution and the storage of beef in vacuum packaging improves the tenderness by ageing without the evaporative weight loss. Vacuum packaging has also advantages in inhibiting aerobic bacterial growth and preventing oxidative rancidity. This type of packaging is not suited for retail use. Due to the lack of oxygen in the package the oxymyoglobin will change its form to deoxymyoglobin which has a purplish-red colour. Low oxygen pressure in the package will cause brown metmyoglobin formation because the rate of oxidation is greater than any enzymic reduction. Vacuum packaged meat of normal pH can be stored up to ten weeks at cold temperatures (Renerre, 1990).

Meat is pre-packaged in modified atmosphere (MAP) including nitrogen, carbon dioxide, oxygen, alone or used in mixtures. In retailing beef meat, the use of inert gases such as nitrogen and/or carbon dioxide is of less interest because they do not enhance the red display colour. The advantage of oxygen in developing a thick layer of oxymyoglobin is desired (Renerre, 1990).

High-oxygen MAP involves a gas mix of up to 80% oxygen (65-80% is most common) and 15-30% carbon dioxide; optionally nitrogen may be added at up to 20%.
The major function of the high levels of oxygen is to maintain the cherry-red meat colour. Due to the high usage levels the oxymyoglobin layer gets more deeply into the meat. This extends the time until the metmyoglobin layer approaches the surface of the meat. A combination of 80% oxygen with 20% carbon dioxide can remain the red colour of the meat for up to 15 days. A possible disadvantage of using high levels of oxygen is the possible development of oxidised colour and taste.
The function of carbon dioxide is to slow down the growth of commonly occurring *Pseudomonas* spp. as well as competing species, thus extending the time until spoilage. Increasingly effective microbial control can be reached up to 25% carbon dioxide. Carbon dioxide is more effective with low levels of initial contamination and also at low temperatures. A possible negative effect is that it promotes the growth of psychotropic lactic acid bacteria. Carbon dioxide is very soluble in meat, therefore enough carbon dioxide has to be added to the gas mix to account for absorption into the meat to maintain effective microbial control.
Nitrogen is an inert gas and is used as a filler gas; it has no direct function in microbial control.
A new and increasing component of the gas mixture is the usage of carbon monoxide (CO). The use of carbon monoxide results in a stable bright-red colour. Carbon monoxide is used in packages with a combination of 60% carbon dioxide and up to 40% nitrogen. The usage of carbon monoxide is allowed up to 0.4%. The main features of this gas mixture are the enhancement of the bright-red colour and a long microbial shelf life due to the high amount of carbon dioxide and the absence of oxygen (Kropf, 2004).

- Light

In general, greater pigment oxidation occurs in meat stored under light versus dark storage conditions (Faustman and Cassens, 1990). White fluorescent lights do not generally cause discolouration, however several studies have shown some detrimental effect. Several studies concluded discolouration under fluorescent light as an indirect effect. The temperature inside the meat package can rise, resulting in growth of spoilage bacteria. Also, a photochemical effect can be the cause of the discolouration. In contrast to white fluorescent light, UV light has an effect on the colour of fresh meat. The myoglobin is oxidised and the meat turns brown after a short exposure. This might be achieved through denaturation of globin. Occasionally a "rainbow-like" discolouration can be found on fresh meat. This is caused by the "prism effect" due to the fibrous character of the meat surface (Renerre, 1990).

- Housing

Vestergaard *et al.* (2000) compared extensively fed young bulls and intensively fed young bulls. It was shown the skeletal muscle characteristics of the extensively reared young bulls changed towards slow contraction, better vascularization and more oxidative metabolism compared to intensively fed tie-stall young bulls. Due to these changes bulls from the extensively system were probably more resistant to muscle fatigue. The pigmentation of the extensively fed bulls was higher but it did not result in more red meat, it did however result in more dark meat (no DFD meat).

2.3.2 Intrinsic factors

- Between species

Colour pigment differ among species; poultry and pork contain about 1 to 3 mg myoglobin per gram wet tissue. Lamb has a brick red colour and contains 4 to 8 mg myoglobin per gram wet tissue and beef, at the age of human consumption, contains around 4 to 10 mg myoglobin per gram wet tissue (Anonymous 1).

According to Atkinson and Follett, beef has the best colour stability and lamb the worst. The colour stability of pork is between that of beef and lamb (Atkinson and Follett, 1973).

- Between animals

Among the intrinsic factors affecting muscle constitution, between animal variability is the least understood. Parallel to chronological age, the concentration of myoglobin in muscle increases and muscles used for locomotion appear darker in colour. Over the period of birth to 24 months of age, a three-phase increment in myoglobin level has been found depending on breed and muscle examined. In meat from older animals the cherry-red layer of oxymyoglobin is thinner and consequently meat colour stability weaker (Renerre, 1990).

Veal contains 1 to 3 mg of myoglobin per gram wet tissue, young beef 4 to 10 mg myoglobin per gram wet tissue and mature beef contains 16 to 20 mg myoglobin per gram wet tissue (Anonymous 1).

- Between muscles

The inter-muscle variation is often directly related to the metabolic and contractile properties. There are three major fibre types: slow-twitch oxidative fibres (SO), fast-twitch glycolytic (FG) and fast-twitch oxidative-glycolytic (FOG). SO fibres are rich in myoglobin, contain a large number of mitochondria, present in high enzymic activity and are present at higher concentrations in "red" muscles. Although relationships between metabolic type and pigment level are well established, except in some heterogenous muscles, this is not the case for colour stability and this point calls for further investigations. For instance, beef M. longissimus dorsi and

M. gluteus medius have similar myoglobin contents and are moderately anaerobic, they differ significantly in post-mortem colour stability and oxygen uptake.
According to Renerre, M. longissimus dorsi, obliquus externus and tensor fasciae latae are the most stable and M. gluteus medius, supra-spinatus, psoas major and diaphragma medialis are the most unstable muscles (Renerre, 1990).

- Ultimate pH and pH rate

The effect of pH on meat colour stability is important from the standpoint of both ultimate pH in post-rigor muscle and the rate of pH decline in the pre-rigor, post-mortem condition. The ultimate pH of normal beef is approximately 5.4 to 5.8. (Faustman and Cassens, 1990). Low pH reduces the stability constant for the haem-globin linkages and myoglobin denaturates at pH values below 5.0. Maintaining a high final pH of the meat by minimising pre-slaughter stress glycolysis, could limit oxidation of the pigments (Renerre, 1990).

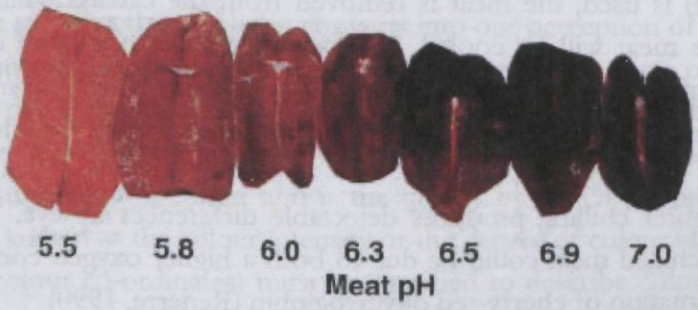


Figure 2.4: Colour change of meat depending on pH (Anonymous 1).

The rate of post-mortem pH decline in the main meat species follows the order poultry > pork > lamb > beef.
Pale, soft, exudative (PSE) meat is formed when the ultimate pH is lower than 5.6. PSE meat is often found in pigs and poultry and sometimes also in beef. Because of the low pH the proteins in the meat will partially denaturate and more light will be scattered, consequently the meat will appear whiter in colour.

If the ultimate pH of the meat is higher than normal abnormal meat called dark, firm, dry (DFD) meat is formed. In high-pH meat, light scattering is reduced and muscle is more translucent at the cut surface and the colour appears darker. Moreover, tissue respiratory activity stays high (mitochondria use the available oxygen) and less oxygen is available for oxymyoglobin formation. The layer of oxymyoglobin is very thin and the purplish-red colour of deoxymyoglobin dominates. The high pH of the meat also results in an earlier spoilage of the meat because of the growth stimulation of bacteria (Monin, 2004).

2.3.3 Extrinsic factors

- Electrical stimulation, hot-boning and chilling rate.

All these factors influence the discolouration indirectly.

Electrical stimulation (ES) of beef carcasses increases the rate of pH drop. Action of ES on meat colour stability during shelf life is still uncertain (Renerre, 1990).

When hot-boning (HB) is used, the meat is removed from the carcass when still warm. After packaging the boneless meat will be cooled. Hot-boning results in a more even colour across large muscles. This might originate from the more homogeneous chilling conditions throughout the whole muscle. A combination of ES and HB is not more efficient than HB alone (Taylor *et al.*, 1980-1981).

Variation in post-slaughter chilling produces detectable differences by eye. The darker overall appearance of rapidly chilled meat could be due to both a higher oxygen consumption rate and an inhibition of the formation of cherry-red oxymyoglobin (Renerre, 1990).

2.4 Measuring the colour of meat

In order to measure the pigment composition of meat, a sample of the meat has to be analysed. This takes some time and to minimise the time necessary it would be useful if this could be automated. A useful method is image processing. The advantages of image processing are evident. It is non-invasive, needing no contact between equipment and the meat. It can be used on film packaged meat without opening the package. Being applied to the whole meat joint, it can ensure that no visible defect is forgotten. There are several methods of image processing which will be discussed in this paragraph.

2.4.1 Background information on colour

Physical properties of colour require a source of light, an illuminated sample and a detector. The light source is described by its spectral energy distribution curve, the sample by its spectral reflectance curve and the detector by its spectral response curve. The combination of these factors provide the stimulus that the brain converts into our perception of colour.

Colour has three quantitatively definable dimensions. "Hue" is the name of the colour and is that quality by which the colour families (e.g. red, green etc.) are distinguished. "Value" is the lightness of colour and is the quality at which lighter and darker colours are distinguished. "Chroma" is the strength of a colour and is the quality by which strong and weak colours are distinguished, also known as the colour intensity or the degree of colour saturation.

Three quantities (colour co-ordinates) must be specified to describe colour. Instrumental colour measurement techniques can be classified by the way in which the light is treated in the measurement process. The three classifications are unaltered light, three or four coloured lights and monochromatic light (light of only one colour). Instruments using three (or four) coloured lights are called colourimeters, whereas instruments using monochromatic light are called spectrophotometers and are capable of measuring the spectral reflectance (or transmittance) curve of a sample.

Colourimeters readings should never be considered to have any absolute significance, but should rather be used for detecting and measuring small colour differences between samples that are nearly alike in colour.

Spectrophotometric reflectance techniques are especially suitable for following either muscle colour changes, or for estimation of myoglobin forms. The samples are not destroyed and the package environment is maintained, so samples can be measured over time. The same location on the cut surface may be measured repeatedly. In contrast to colourimeters, spectrophotometers can be used to obtain absolute values of the colour co-ordinates for a single sample as well as for measuring colour differences between samples. Spectrophotometric reflectance techniques are not commonly used in the food industry but mostly in research (AMSA, 1991).

2.4.2 Instrumental colour evaluations systems

- Munsell colour solid

A.H. Munsell was one of the first (1905) to describe colour in terms of 3-D colour solid.

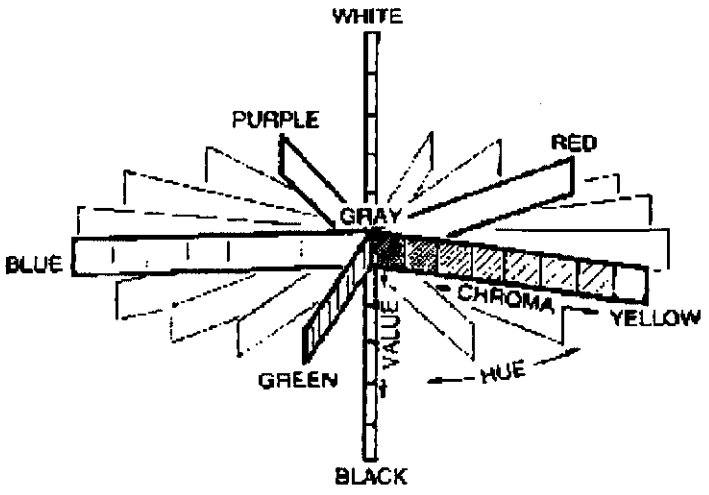


Figure 2.5: The Munsell colour solid (AMSA, 1991).

Colour is described in terms of three attributes; hue = H, value = V and chroma = C. There are five principal hues (red, yellow, green, blue and purple) equally spaced around the circle at the base of the colour solid. The value notation in the vertical axis indicates the degree of lightness or darkness of a colour. Zero is absolute black and 10 is absolute white.

Disc colourimetry was one of the first instrumental methods to quantitate colour of an unknown sample. Colour may be expressed in Munsell notation or converted to CIE (Commission Internationale de l'Eclairage, also International Commission on Light) tristimulus values. Due to the unavailability of instruments that directly read out tristimulus values, disc colourimetry has not been used widely in recent studies (AMSA, 1991).

- CIE colour solid

Most colours can be made by the appropriate mixture of the light-primary colours green, red and blue. Not all colours can be made in this way and some colours only can be made when red light is subtracted from the blue/green mix. This leads to negative values for red light. This can cause problems when calculations are done. To avoid these problems with negative values, the CIE developed the XYZ primary system to describe colour in numerical terms without the use of negative numbers. XYZ can be calculated from the experimental red, green and blue primaries.

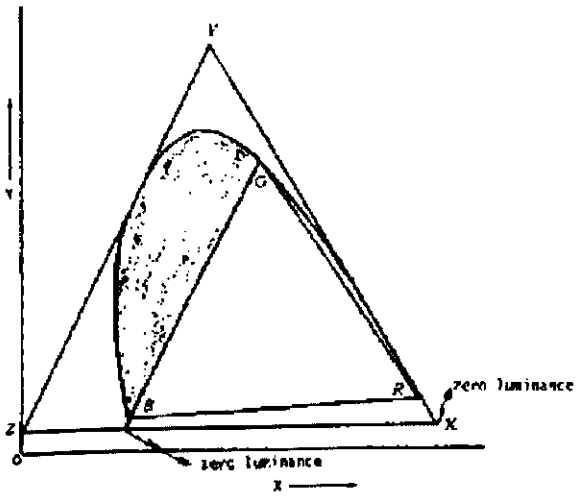


Figure 2.6: The complete XYZ triangle of the CIE system (AMSA, 1991).

In figure 2.6 the relation between the XYZ triangle and the red, green and blue values is shown. The shaded area represents the negative R-values. In Tristimulus colourimetry X, Y and Z filters are used. The filters are designed, so the energy output of the light source, modified by the filter, creates the same response in the photo detector as in the human eye (ASMA, 1991).

- Hunter colour solid

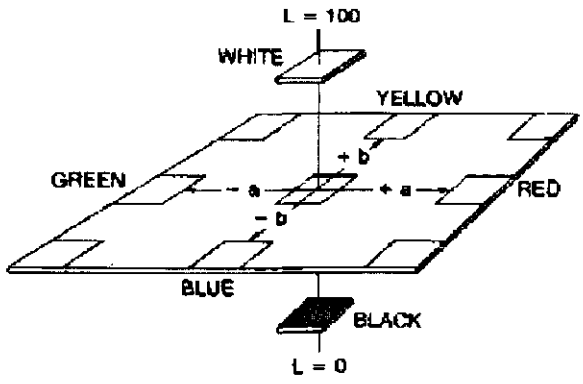


Figure 2.7: Hunter L a b colour solid (AMSA, 1991).

The lightness axis in the centre of the solid has a scale from 0 to 100, where 100 is absolute white. Positive a-values are red and negative a-values are green. Positive b-values are yellow and negative b-values are blue. Hunter L a b colour solid is preferred above the CIE X, Y, Z system and widely used because of its uniformity and easy to use Hunter colourimeters (AMSA, 1991).

2.4.3 Method of colour analysis used in this study

Spectrophotometers and tristimulus colourimetry are used for samples with uniform surfaces and colours. The measured colour is a single value in XYZ or L a b colour scale. For foods with different colours or non-uniform surfaces such as meat, a single value is not a good representation of the actual colours. Samples can be chopped or blended to get an average colour but the original colour and appearance of the sample is usually lost.

In this research, computer vision is used. Computers are getting more used in the production environment. In computer vision, an image of the sample is digitised into pixels, containing levels of the three primary colours (red, green and blue = RGB colour system). R-, G- and B- values are expressed as a value varying between 0 and 255. By using image processing techniques one can identify and classify an object based on colour and appearance. One can also provide a quantitative description of all the colours of the sample. With this procedure samples with varying colours, different shapes, sizes and surface textures can be analysed (Luzuriaga and Balaban, 2002).

3 Materials and Methods

3.1 Meat samples

In this research rib eye was used. Rib eye is normally cut out of the small ribs (5th till 7th rib counted from the head) and the large ribs (8th till 11th rib). These parts of the beef consist out of different muscles. The most important are Musculus (M.) serratus, M. Latissimus dorsi, M. longissimus dorsi, M. spinales et semispinales, M. dorsi et cervicis and M. multifudus.
(Bergström, 1974). The meat was kindly provided by ProMessa located in Deventer (NL). The nutritional value and the information belonging to the meat can be found in appendix 2.

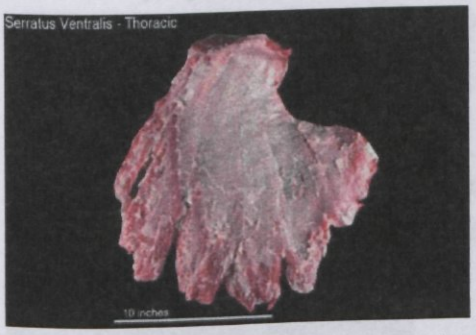


Figure 3.1 : Serratus ventralis – thoracic (Anonymous 2).

3.2 Experiment design

3.2.1 Myoglobin content and related pigments

Determination of the total myoglobin content and of the proportions of oxymyoglobin, deoxymyoglobin and metmyoglobin in meat, is based on the different absorbance spectra of these molecules when in solution. The method used in this research is according to Carlez *et al.* (1995). Absorbance measurements are conducted after pigment extraction from meat samples. This method measures the heme pigment content of the total meat sample, in contrast to reflectance methods, which indicate the pigment content at the surface of the meat.

In 2004 Tang *et al.* (2004) published revised equations how to calculate the proportions of oxymyoglobin, deoxymyoglobin and metmyoglobin. These are more reliable than the original ones by Krzywicki (Carlez *et al.*, 1995).

$$\% \text{ Deoxymyoglobin} = (-0.543\ R_1 + 1.594\ R_2 + 0.552\ R_3 - 1.329) * 100$$
$$\% \text{ Oxymyoglobin} = (0.722\ R_1 - 1.432\ R_2 - 1.659\ R_3 + 2.599) * 100$$
$$\% \text{ Metmyoglobin} = (-0.159\ R_1 - 0.085\ R_2 + 1.262\ R_3 - 0.520) * 100$$

$$\text{Total myoglobin (mmol/l)} = A^{525} / \text{MAC } 525$$

MAC^{525} (= 7.6) (Krzywicki, 1982) is the milli molar coefficient of myoglobin at the isobestic point (525 nm) of its three forms and R_1, R_2, R_3 are respectively the absorbance ratio A^{582} / A^{525}, A^{557} / A^{525}, A^{503} / A^{525}.

For extraction of the three forms of myoglobin the outer layer of the meat is sliced off to a depth of 3 to 4 mm. When light falls on a meat surface it is not only reflected from the uppermost layer but penetrates to a tissue depth of 3 to 4 mm (Krzywicki, 1979).

Protocol: Slice 2 gram out off the surface of the meat to a depth of 3 to 4 mm. Homogenise the meat with 20 ml of 0.04 mol/l Na/K phosphate buffer, pH 6.8, using an Ultraturrax (Janke & Kunkel IKA Labortechniek T25) homogenizer at approximately 10,000 rpm and room temperature for 20 s. After standing for 1 hour in an iced bath, the homogenate was centrifuged at 10,000 x g and 10-15 °C for 30 min. The supernatant was filtered through a Mackery-Nagel MN 619 ¼ paper and the volume is brought up to 25 ml with the same phosphate buffer. Low temperature has to be maintained to avoid turbidity. Filter the supernatant on a 0.25 µm Millipore filter before spectrophotometry. Absorbance of the clear supernatant has to be measured at 503, 525, 557 and 582 nm (Analytik Jena AG Spekol 1300).

3.2.2 *Colour analysis*

The colour of the meat was analysed by a computerprogram called Peer v11 (personal communication 1). The meat packages (without top foil) are put in a closed cabinet with constant illumination shielded by fluorescent tubes. A picture of the meat was taken with a Sony 3 CCD camera (which is calibrated with Colorchecker® of Gretag Macbeth™, see also appendix 3). In Peer v11 a circle with a diameter of 20 mm was drawn over the picture after which the program calculates the average red, green and blue (RGB) values and the standard deviation of each. Four measurements are taken from each picture taking care fat was left out the measuring circle. The RGB values and their standard deviations are the average of 4 measurements.

Figure 3.2: Cabinet in which the pictures of the meat were taken.

3.2.3 Approach

Pro-Messa provided 80 pieces of rib eye. A small piece was cut out of each piece of meat to measure the pH, every rib eye is photographed and the RGB values are measured. The meat samples are packaged in 1500 cm^3 trays. Meat samples 1 to 40 were packaged in modified atmosphere (60% O_2, 30% CO_2 and 10% N_2) and covered with coated PET. Meat samples 41 to 80 were packaged aerobically with polyethylene stretchfoil (Suntec S, permeability CO_2 90,000; O_2 23,000 ml/m^2/ bar/ day). Samples 1 to 20 and 41 to 60 were stored at 2°C and samples 21 to 40 and 61 to 80 were stored at 7°C in the cold storage room (see also table 3.1).

Table 3.1: Design of the meat experiment.

Meat sample	Type of atmosphere	Storage temperature (°C)
1 – 20	Modified atmosphere	2
21 – 40	Modified atmosphere	7
41 – 60	Air	2
61 – 80	Air	7

On each day of sampling a photo was taken when the meat was taken out of storage and the RGB value was measured. A small piece of meat was cut off for pH measurement and the myoglobin content and the percentage of the three forms of myoglobin was determined as described in paragraph 3.2.1.

pH measurement

The pH was measured by mixing meat with deionized water in a 1:3 ratio using the Ultraturrax for 10 s at 10,000 rpm, after which the pH of the solution is measured.

4 Results and discussion

All the results discussed in this chapter, the original measurement results and all digital photo's have been archived and can be reviewed on the CD, which is attached to this report.

4.1 RGB measurement: point measurement – surface measurement

In order to reduce the standard deviation of the RGB measurement, the point measurement (measuring a precise co-ordinate) was changed to surface measurement.

When using a point measurement, four precise co-ordinates are measured and averaged. Before starting this research it is known the standard deviation of this method is substantial. To lower the standard deviation of the RGB measurement it was proposed to measure four times a small surface and average the results. A circle with a diameter of 20 mm can be drawn in the picture taken form the meat. The computerprogram calculates the average R, G and B value and the accompanying standard deviations. When this is done four times on a piece of meat, the standard deviations of the four measurements are averaged. The results are shown in the next table. All measurements were done at day 0 of the experiment before the meat was packaged to exclude effects of package atmosphere.

Table 4.1: Initial RGB measurement values according to point and surface measurement method.

	Point measurement			**Surface measurement**		
Sample	**R**	**G**	**B**	**R**	**G**	**B**
7 10/5	54 ± 9	21 ± 3	17 ± 3	56 ± 8	19 ± 4	17 ± 4
9 10/5	50 ± 8	20 ± 3	17 ± 6	54 ± 8	20 ± 4	17 ± 5
22 10/5	67 ± 9	26 ± 3	24 ± 5	66 ± 8	24 ± 5	21 ± 6
31 10/5	58 ± 20	22 ± 10	18 ± 11	58 ± 8	23 ± 4	21 ± 5
48 10/5	49 ± 10	18 ± 3	15 ± 4	49 ± 7	18 ± 3	16 ± 4
56 10/5	49 ± 7	21 ± 4	19 ± 5	47 ± 5	18 ± 3	16 ± 4
67 10/5	63 ± 11	24 ± 6	21 ± 8	65 ± 7	23 ± 4	20 ± 4
77 10/5	55 ± 9	20 ± 3	16 ± 5	65 ± 9	24 ± 5	17 ± 6
Average	**55 ± 10**	**21 ± 4**	**18 ± 6**	**58 ± 8**	**21 ± 4**	**18 ± 5**

From the results in table 4.1 it is clear that the standard deviations of the surface measurement method are better than that of the point method. All standard deviations except the one of the G-value is lower when the surface measurement method is used.
It is assumed red is the most important indicator in this research because changes in the meat also result in a different red colour. The standard deviation of the R-value is still quite large when the surface measurement method is used, because meat has a biological variation in red colours between muscles but also inside muscles. The surface measurement method has a higher R-value (58) than the point measurement (55). It is possible a more bright-red piece of meat was included in the measuring circle when measuring the RGB.
The surface measurement has lower standard deviations than the point measurement, but there are still some shortcomings. When a piece of meat is used for a shelf life test, the RGB of the meat is measured at the beginning and end of the test. To get a reliable result you have to measure the RGB both times at the same co-ordinates. This is very difficult, when taking a photograph at the end of the test the package containing the meat has to be situated at the same place as when the photograph was taken at the beginning of the test. This is very precise because a small displacement in the picture gives completely different RGB measurements. Another problem are the pieces of fat in the meat, these pieces have a whitening effect on the RGB measurement. It is not always possible to exclude fatty parts. There is a possible solution for these problems. During this research it appeared it is possible to measure the RGB with a computer program designed at Agrotechnology & Food Innovations
(personal communications 1). It was not yet possible to work with this program because of practical reasons. This program measures eight different colours of red, its surface and is able to ignore fat. From the red colours and the surface of each colour it is possible to calculate the average RGB value of the whole piece of meat. This program might be more accurate because it takes a lot of measuring points and not only the four points that are used in this research.

4.2 The absorption spectrum of the sodium/potassium phosphate buffer

While conducting experiments it appeared the results for the isolation of the three confirmations of myoglobin where not realistic. It became apparent the buffer had an own absorption spectrum, as shown in the figure below. Before the formulas of paragraph 3.2.1 can be used the absorption of the buffer has to be subtracted from the absorption of the sample.

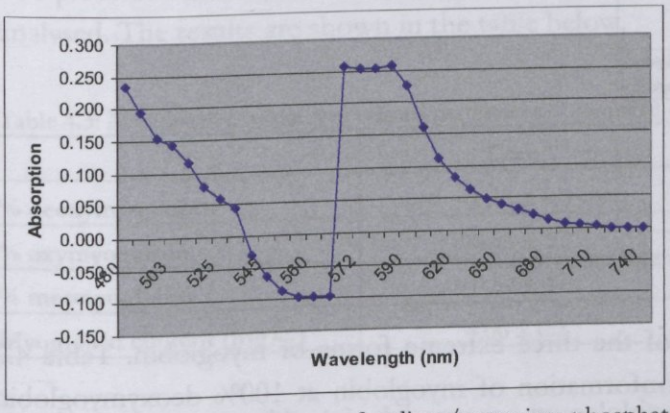


Figure 4.1: Absorption spectrum of sodium/potassium phosphate buffer.

4.3 Extremes of deoxy-, oxy- and metmyoglobin and their corresponding red, green and blue values

In this experiment 100% deoxymyoglobin, oxymyoglobin and metmyoglobin were artificially made to measure the extreme RGB values. The method described in the "Guidelines for meat color evaluation" by the American Meat Science Association (AMSA, 1991) were used to prepare the meat. The method and pictures of the meat can be found in appendix 4. Two samples from each piece of meat were analysed.

Table 4.2: Analysis results of the extremes of myoglobin.

	100% deoxymyoglobin		**100% oxymyoglobin**		**100% metmyoglobin**	
	Sample 1	Sample 2	Sample 1	Sample 2	Sample 1	Sample 2
% deoxymyoglobin	- 6.9	-6.5	-10.1	-14.3	-0.04	0.1
% oxymyoglobin	77.1	97.7	115.2	123.7	0.7	0.3
% metmyoglobin	29.3	8.5	-5.8	-10.4	99.1	99.5

From table 4.2 it is evident that deoxymyoglobin can not be quantified with the method of Krzywicki. During the procedure the deoxymyoglobin is instantaneously oxidized to form oxymyoglobin. The results for oxymyoglobin in the rest of this research can be assumed to be the total for deoxymyoglobin and oxymyoglobin.

Table 4.2 shows that the method is suitable for the quantification of oxymyoglobin and metmyoglobin. A possible explanation for exceeding 100% as a result in the quantification of

oxymyoglobin is that the calculated percentage of oxymyoglobin is the total of deoxymyoglobin and oxymyoglobin.
The method of Krzywicki used in this research is suited for the quantification of metmyoglobin, the percentage of metmyoglobin almost reaches 100%.

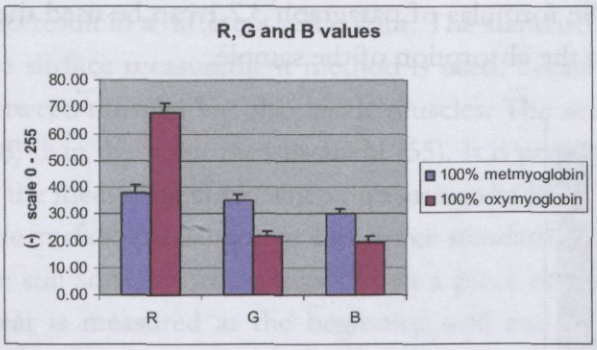


Figure 4.2: R-, G- and B-value of 100% deoxy-, oxy- and metmyoglobin.

Figure 4.2 shows the R, G and B values of the three extreme forms of myoglobin. Table 4.2 showed it was not possible to keep the conformation of myoglobin at 100% deoxymyoglobin during its isolation. The pictures necessary to measure the RGB values are taken a few seconds after opening the package containing the 100% deoxymyoglobin meat to prevent oxidation. The R, G and B values of 100% deoxymyoglobin in this figure are therefore reliable.
100% oxymyoglobin has the highest R-value and, as can be seen in appendix 4, this corresponds to the bright red colour of the meat. 100% metmyoglobin has a lower R value, this suggest a decrease in the R value results in a more brown colour of the meat as can be seen in the picture in appendix 4. It is not possible to make a distinction between 100% metmyoglobin and 100% deoxymyoglobin by looking to the R-value, the B value is also not suited but it is possible to distinguish the two by looking at the G-value, this is higher for 100% metmyoglobin.

4.4 Repeatability of meat's myoglobin content, it related pigments and the recovery of myoglobin

4.4.1 Repeatability of meat's myoglobin content and it's related pigments

The repeatability of the determination of myoglobin and its related pigments were assessed using one piece of meat. From this piece of meat, three core samples and four surface samples were analysed. The results are shown in the table below.

Table 4.3: Myoglobin content and related pigments of one piece of meat.

	Core	Surface
% deoxymyoglobin	-1.23 ± 1.0	-3.86 ± 1.1
% oxymyoglobin	90.49 ± 3.7	86.67 ± 8.4
% metmyoglobin	10.39 ± 3.0	16.68 ± 8.0
Myoglobin content (mg/g)	4.06 ± 0.2	4.01 ± 0.1

As can be seen in table 4.3, the average myoglobin content of the core samples is 4.06 ± 0.2 mg/g. The surface samples have an average of 4.01 ± 0.1 mg/g myoglobin. The myoglobin content and its deviation of the core and surface samples does not show a large difference. The deviation between the core and surface can be explained as a fault in the method of analysis, also in literature it says myoglobin is not evenly distributed throughout the meat. Swatland (Swatland, 1989) states myoglobin in pork muscle is more present in the aerobic part of the muscle. Polasek and Davis (Polasek and Davis, 2001) looked into the myoglobin content of whale and dolphin muscle, they found the interior of the muscle has a higher concentration of myoglobin than the outer layer of the muscle. No literature is found to state this about beef meat, but it can be assumed it will be the same concerning to beef.

As stated above the core samples have an average myoglobin content of 4.06 ± 0.2 mg/g and the surface samples have an average of 4.01 ± 0.1 mg/g myoglobin. McKenna *et al.* (2005) analysed the amount of myoglobin at the meat surface of beef and found an average of 4.6 mg/g. This is close to the measurements of the piece of meat discussed in this paragraph, but also to the measurements discussed in paragraph 4.5 and shown in the CD attached to this report. The amount found by McKenna has to be used as an indicator, because there are a lot of factors that can influence the amount of myoglobin like race and feed.

The average oxymyoglobin content in the core samples is 90.49 ± 3.7% with a maximum. The surface samples have an average oxymyoglobin content of 86.67 ± 8.4%. Theoretically it is not possible that oxymyoglobin is present in the core of a piece of meat. Oxygen does not migrate to the centre of the meat, therefore the myoglobin stays in the deoxymyoglobin conformation. As described in paragraph 4.3, it is not possible to isolate deoxymyoglobin with the method

described in the article of Carlez *et al.* (1995). The measured oxymyoglobin in the core is obtained from the deoxymyoglobin that has bound oxygen during mixing with the Ultraturrax.
The results of the surface samples show a quite large deviation in the amount of oxymyoglobin. It is possible the confirmation from oxymyoglobin to metmyoglobin does not take place at the same rate at every place in the muscle.

The average metmyoglobin content of the core samples is 10.39 ± 3.0%. The surface samples contain an average metmyoglobin content of 16.68 ± 8.0%. It is possible the change in conformation to metmyoglobin does not take place at the same rate throughout the meat.

4.4.2 Recovery of myoglobin

The goal of this experiment is to investigate the accuracy of the method described by Carlez (see paragraph 3.2.1). McKenna *et al.* (2005) found a myoglobin content of 4.6 mg/g in bovine muscle. In this experiment 5 mg myoglobin (Sigma M0630-1g, >99%) per g meat was dissolved in sodium/potassium phosphate buffer (paragraph 3.2.1) and the absorbance was measured at 503, 525, 557 and 582 nm, after which the amount of myoglobin can be calculated. The experiment was performed in duplicate.
After calculation 3.6 respectively 3.7 mg/g myoglobin was found in the solution. This is a recovery rate of 72 and 74%. It was not possible to get a recovery rate of 100%, because not all the myoglobin could be dissolved in the buffer. Therefore it is not possible to give a statement about the accuracy but regarding the results it will be likely that if all the myoglobin was suspended the 100% recovery rate would be reached.

4.5 Meat experiment with different storage conditions

Meat was packaged either in modified atmosphere or aerobically and stored at 2°C or 7°C. During a period of 8 days the meat was photographed every day and samples were taken for pigment analysis. The original photos and results can be looked up in the CD which is attached to this report. This experiment resulted in much data, in this paragraph first the correlation coefficient will be shown for all the data after which the different data will be discussed.

At the start of this experiment the RGB of each piece of meat was measured, when the meat was analysed the RGB was measured again. The R, G and B in this paragraph are the values of the moment of analyses minus the values of day 0. A positive value means the meat has more of a particular parameter than at day 0 and a negative value means a decrease in a particular parameter than at day 0.

Table 4.4: Correlation coefficients of the data obtained from the meat experiment.

Correlation coefficient				
	MAP		Air	
	2°C	7°C	2°C	7°C
Time - R	**0.795**	0.486	**0.718**	**0.729**
Time - G	0.081	**0.705**	0.369	0.641
Time - B	0.010	**0.740**	0.000	**0.892**
Myoglobin - red	0.008	0.417	0.026	0.000
Myoglobin - green	0.141	0.461	0.222	0.059
Myoglobin - blue	0.226	0.513	0.152	0.011
% deoxymyoglobin - red	0.339	0.000	0.537	0.671
% oxymyoglobin - red	0.493	0.673	0.622	0.235
% metmyoglobin - red	0.527	0.591	0.409	0.184
% deoxymyoglobin - green	0.176	0.014	0.039	0.181
% oxymyoglobin - green	0.198	0.593	0.351	0.001
% metmyoglobin - green	0.187	0.456	0.480	0.198
% deoxymyoglobin - blue	0.040	0.252	0.012	0.418
% oxymyoglobin - blue	0.043	0.353	0.002	0.117
% metmyoglobin - blue	0.038	0.138	0.015	0.144
All R - all % oxymyoglobin	0.500	0.500	0.500	0.500
Time - average % oxymyoglobin	0.518	0.584	**0.884**	0.461
Time - average % metmyoglobin	0.529	0.179	**0.745**	0.147
average % metmyoglobin - average % oxymyoglobin	**0.924**	**0.771**	**0.834**	0.157
Time - myoglobin	0.076	0.432	0.146	0.066

4.5.1 *Colour changes of the meat in time*

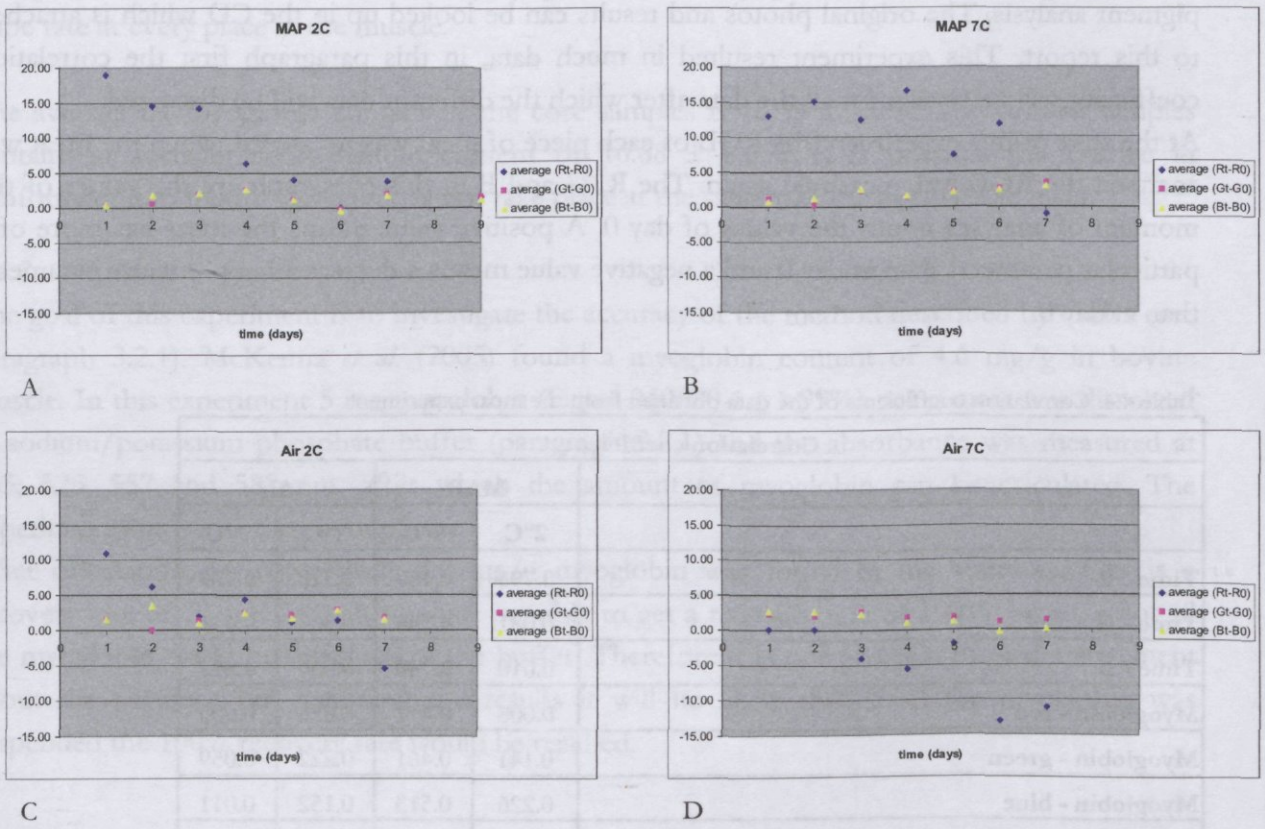


Figure 4.3: Change in R-, G- and B-value in time (A= MAP 2°C, B= MAP 7°C, C= Air 2°C, D= Air 7°C).

On each day of analysis two pieces meat of each type of packaging and storage temperature were analysed. The R-, G- and B-values of the two pieces of meat were averaged.

Figure 4.3 shows a decrease in the redness of the meat during time. The meat samples packaged in modified atmosphere show an increase in redness in the first few days of the experiment. The gas mixture in the modified atmosphere package contains 60% oxygen, this causes the meat to bloom. Modified atmosphere packaged meat samples remained red for the duration of the experiment.

Meat samples packaged aerobically show a rapid decrease in redness. The samples stored at 7°C did show a more rapid decrease in the R-value. Meat stored at 2°C becomes less red than at day 0 after approximately 6 days and meat stored at 7°C after approximately 2 days. Lower temperatures apparently slow the process of red-colour loss down.

The green and blue values of all samples do not show a large change in time.

The pictures taken from all the meat samples show the meat changes colour to brown in time (see attached CD). In time the R-value decreases and the G-value does not show a large variation. This corresponds with the theory that when red and green are mixed together in the right proportion the colour brown is formed.

4.5.2 *Myoglobin concentration in relation to the colour of meat*

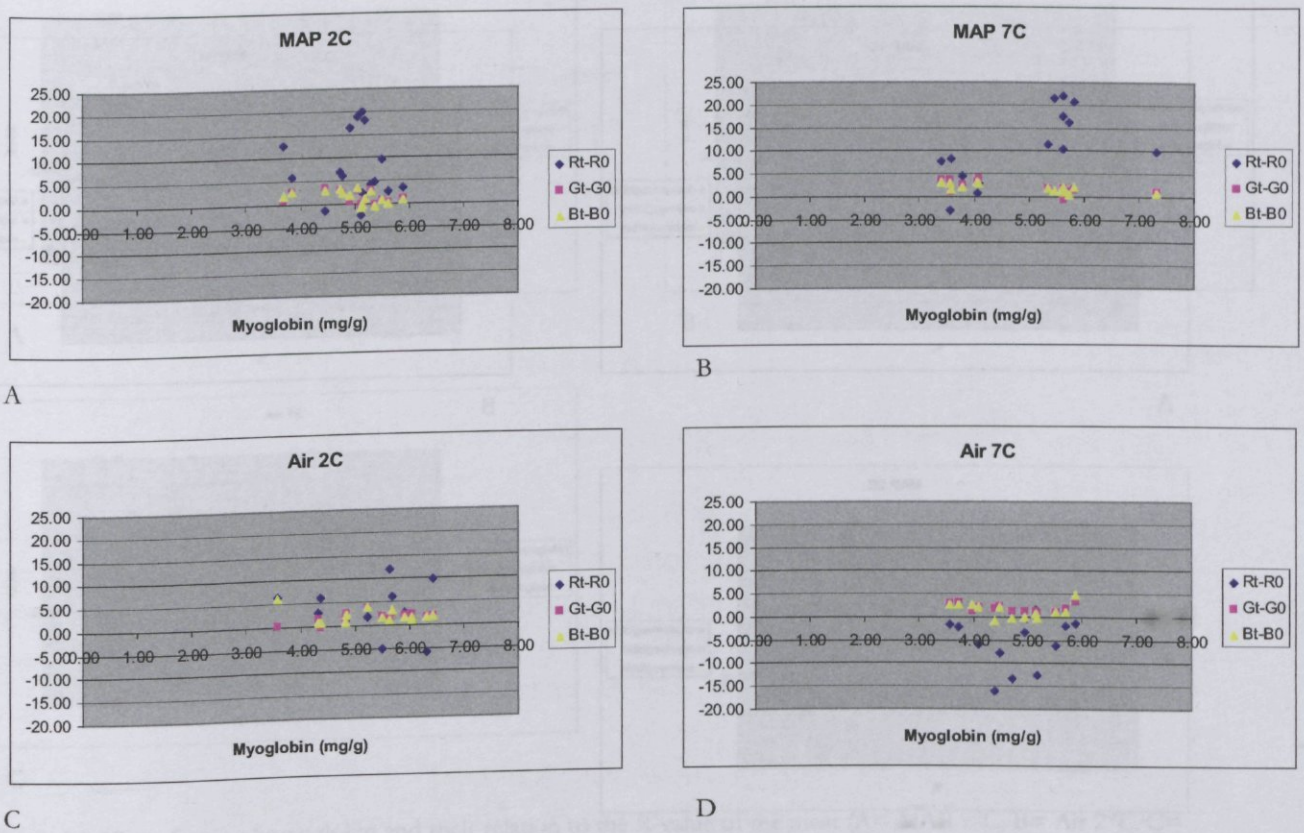


Figure 4.4: Myoglobin concentration in relation to the colour of the meat (A= MAP 2°C, B= MAP 7°C, C= Air 2°C, D= Air 7°C).

Figure 4.4 clearly shows that there is no relation between the total myoglobin content in the meat and the colour of the meat.

4.5.3 *Three forms of myoglobin and their relation to the R, G and B of the meat*

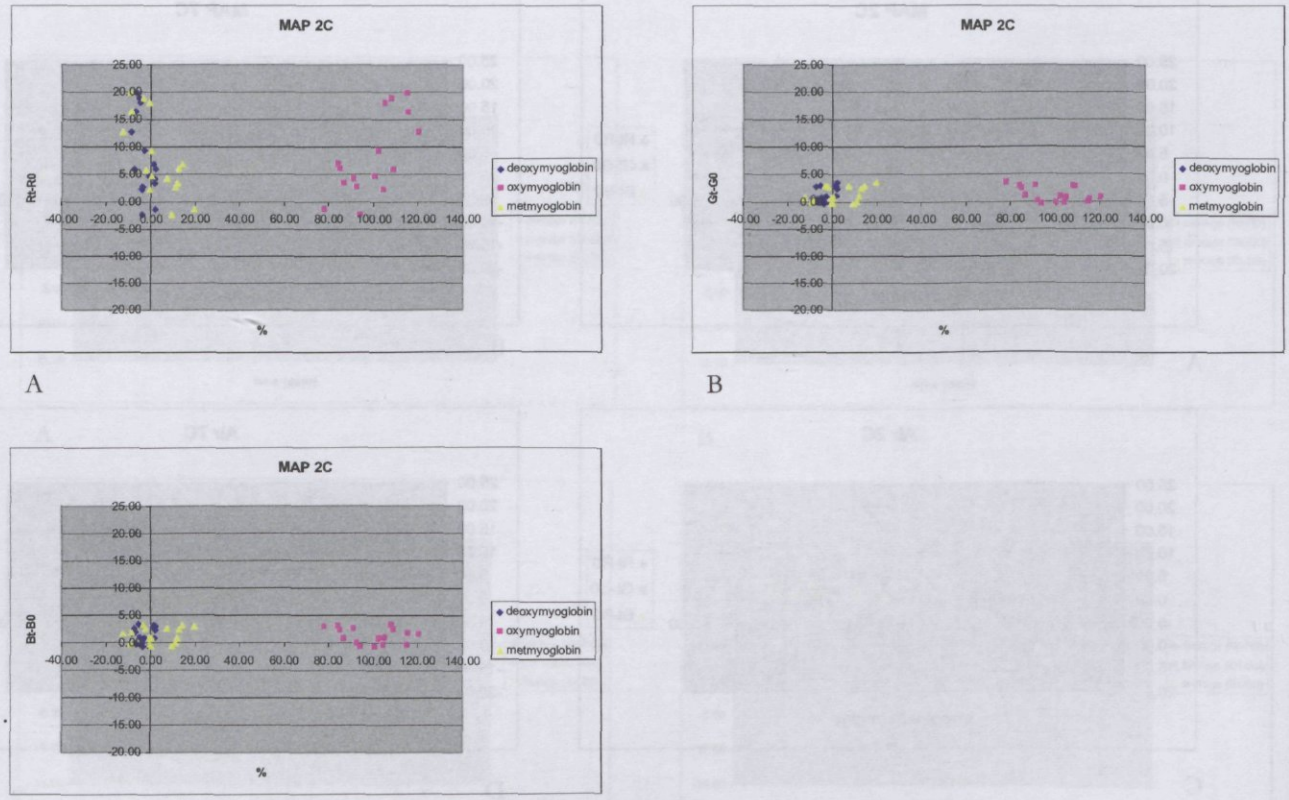
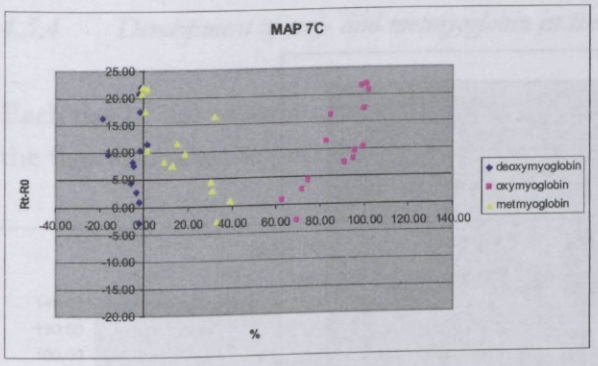


Figure 4.5: Three forms of myoglobin and their relation to the R-, G- and B-value of the meat.

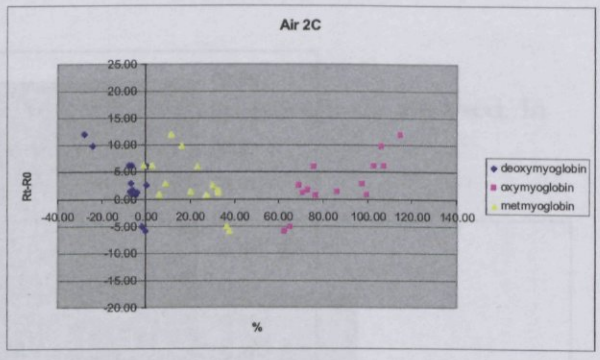
From figure 4.5 it is apparent that there is a relationship between the percentage of oxy- and metmyoglobin pigments and the average R-value of the meat. The R-value rises sharply when the oxymyoglobin content is more than 80%. A rise in the percentage of metmyoglobin results in a drop of the R-value. The results for deoxymyoglobin are not reliable as explained in paragraph 4.3.

The figures 4.5 B and C show there is no relation between the three forms of myoglobin and the G- or B-value. In these figures a cloud of measuring points can be seen.

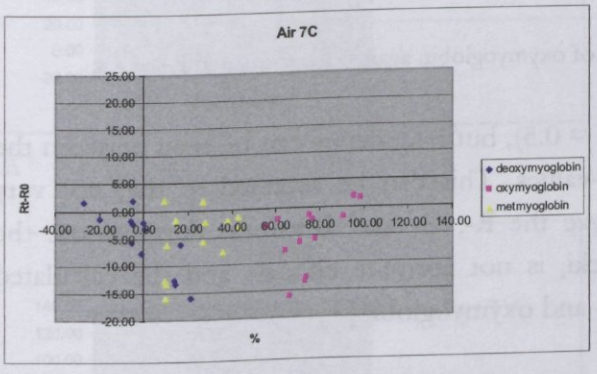
The figures of G- and B- value for the other samples are not shown here, for those figures I refer to the attached CD. The other figures for the R-value are shown on the next page.



A



B



C

Figure 4.6: Three forms of myoglobin and their relation to the R-value of the meat (A= MAP 7°C, B= Air 2°C, C= Air 7°C).

The relation described in the previous page between oxy-, metmyoglobin and the R-value of the meat also applies for the other packages and temperatures. A rise in the R-value results in a rise in the percentage of oxymyoglobin and a drop in the percentage of metmyoglobin. Although, there is a trend the correlation is far from perfect, as can be seen from the correlation coefficients (R^2 0.235 to 0.673).

The relation between oxymyoglobin and the R-value is clarified in figure 4.7. All the measured R- values and percentage oxymyoglobin of the different types of packaging and storage are displayed in this figure.

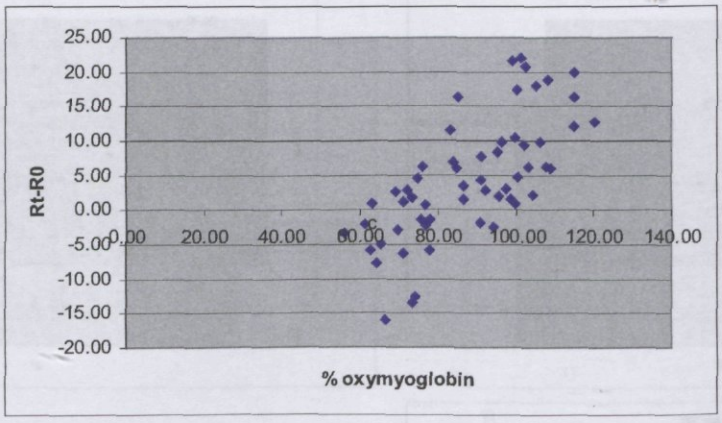


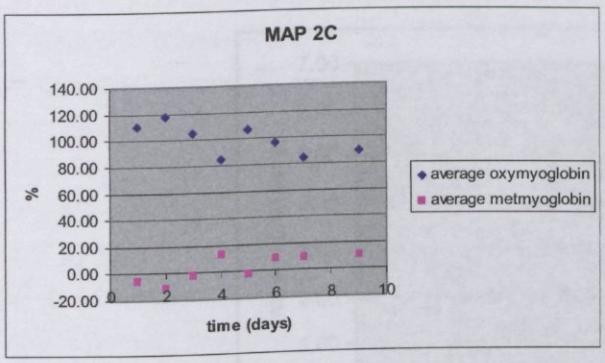
Figure 4.7: All measured percentages of oxymyoglobin against the measured R-values.

Figure 4.7 shows a large spread in the results ($R^2 = 0.5$), but a tendency can be seen between the percentage oxymyoglobin and the measured R-values. This can be ascribed to the, not very accurate, method used in this research to analyze the R-, G- and B-values. Furthermore the pigment analysis, using the method of Krzywicki, is not accurate enough and the calculated oxymyoglobin percentages are the total of deoxy- and oxymyoglobin.

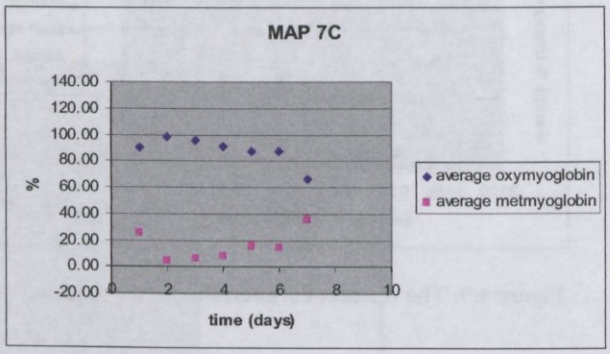
In the research described by McKenna (McKenna *et al.*, 2005) instead of RGB, L*a*b* was measured. a* - values are an indication of the redness. The measurements of McKenna *et al.* show a distinct relation between the a*-value and the amount of met- and oxymyoglobin (R^2 0.9 and -0.97).

4.5.4 *Development of oxy- and metmyoglobin in time*

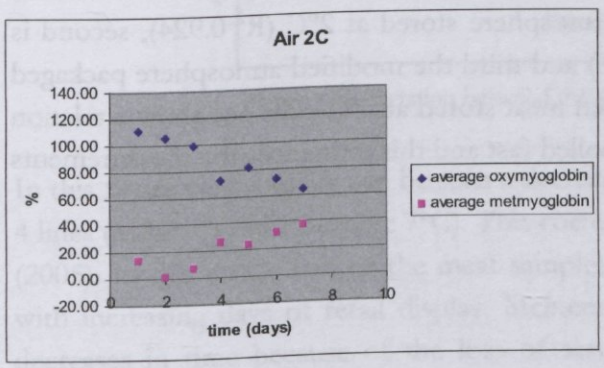
Each day of the experiment two packages of each type of packaging and storage are analysed. In the figures below the percentages of those two samples are averaged.



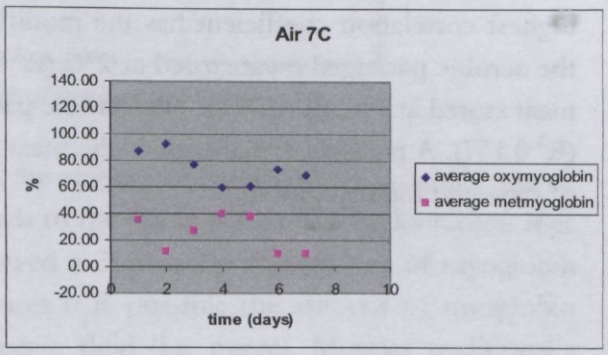
A



B



C



D

Figure 4.8: Development of oxy- and metmyoglobin in time (A= MAP 2°C, B= MAP 7°C, C= Air 2°C, D= Air 7°C).

Figure 4.8 shows the same tendency; a decrease in the percentage of oxymyoglobin results in an increase in metmyoglobin. The decrease in the percentage of oxymyoglobin is less sharp in the MAP packaged samples than in the aerobically packaged samples. The modified atmosphere (containing 60% oxygen) assures that the equilibrium between oxy- and metmyoglobin remains towards the oxymyoglobin form.

Hood (Hood, 1980) stated that the rate of metmyoglobin accumulation increases with increased temperature. In the figures 4.8 A and B of the meat packaged in modified atmosphere this is also observed. The percentage of metmyoglobin in meat stored at 7°C rises faster than when the meat is stored at 2°C. It is not possible to conclude this about the aerobic packaged samples, because the percentage metmyoglobin of the sample stored at 7°C has a peculiar development. This can be the result of measuring faults caused by spoilage products of bacteria growth.

The relation between the percentage oxymyoglobin and metmyoglobin is also shown in the next figure.

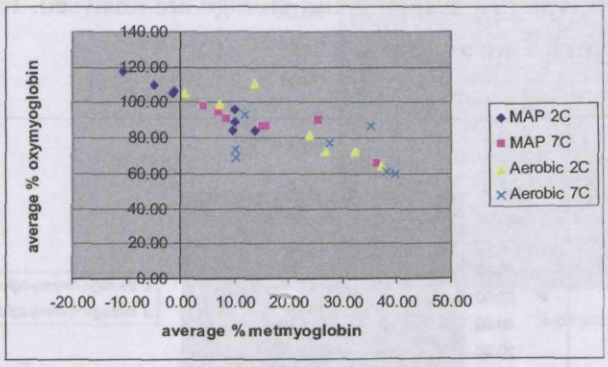


Figure 4.9: The relation between the percentage met- and oxymyoglobin.

In figure 4.9 the relation between the percentage metmyoglobin and oxymyoglobin is clearly seen. When the percentage of oxymyoglobin drops the percentage of metmyoglobin rises. The highest correlation coefficient has the modified atmosphere stored at 2°C (R^2 0.924), second is the aerobic packaged meat stored at 2°C (R^2 0.838) and third the modified atmosphere packaged meat stored at 7°C (R^2 0.771). The aerobic packaged meat stored at 7°C does not show a relation (R^2 0.157). A possible explanation is the meat is spoiled fast and this influences the measurements of oxy- and metmyoglobin.

4.5.5 *The relation between the amount of myoglobin and time*

Each day of the experiment two packages of each type of packaging and storage are analysed. In the figures below the amount of myoglobin of those packages is averaged.

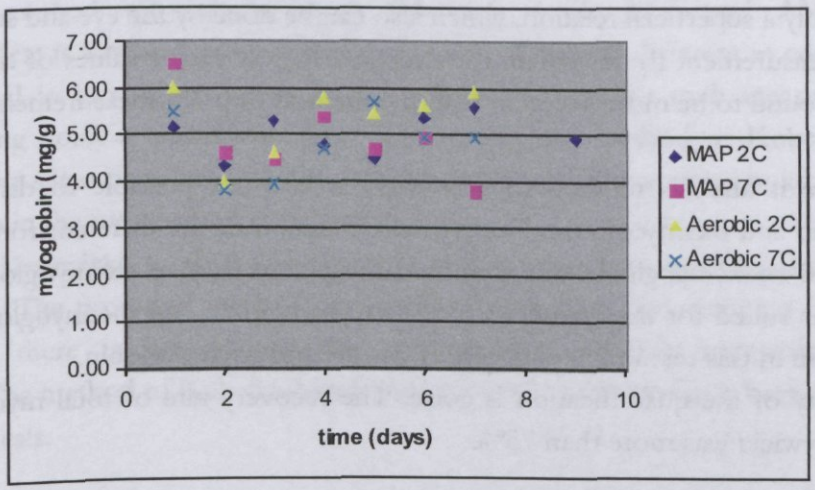


Figure 4.10: Relation between the amount of myoglobin and time.

In this figure very roughly can be seen a decrease in the amount of myoglobin in time of 2 out of 4 lines (MAP 7°C and Aerobic 7°C). This corresponds to the findings reported by McKenna *et al.* (2005). In this article half of the meat samples showed a decrease in the amount of myoglobin with increasing days of retail display. McKenna states it is possible the amount of myoglobin decreases in time because of the loss of sarcoplasmic fluid (i.e. purge). Muscles traditionally characterized by large losses in purge during retail display showed a large decrease in myoglobin content, but large standard errors prevented detecting significant differences in myoglobin content. In this research the purge loss was not measured, but purge was detected visibly.

5 Conclusions

It was not possible to relate beef colour to myoglobin using a digital camera and computer software.

R-, G- and B-values can be used as an indication to estimate the pigment composition of the meat. This is only a superficial relation, which also can be done by the eye and subjective scales. The surface measurement to determine the averaged R-, G- and B-values of meat tested in this research were found to be more accurate than the method of point measurement used before.

With the pigment analysis method of Krzywicki it was not possible to distinguish between deoxymyoglobin and oxymyoglobin. During the extraction of the different forms of myoglobin out of the meat, deoxymyoglobin was instantaneously converted to oxymyoglobin. Nevertheless the method was suited for the quantification of oxymyoglobin and metmyoglobin. The amount of oxymyoglobin in this research is the total of deoxy- and oxymyoglobin.
The repeatability of the quantification is good. The recovery rate of total myoglobin using the method of Krzywicki was more than 75%.

After packaging the modified atmosphere packed meat bloomed, the 60% oxygen in the gas mixture caused the pigment equilibrium to shift towards oxymyoglobin. In all experiments the R-value decreased in time. Meat packaged in modified atmosphere remained red during the experiment. The R-value of aerobic samples declined fastest and the R-value became less than at day 0 after approximately 2 (7°C) and 6 (2°C) days. A lower storage temperature results in a longer colour stability of the meat. In none of the experiments did the G- or B-value change considerably much in time.

In all experiments the percentage oxymyoglobin and metmyoglobin showed a tendency with the corresponding R-values. A rise in the percentage of oxymyoglobin results in a rise of the R-value. The percentage metmyoglobin showed the opposite relation. No relation could be found between the G- or B-value and the percentage of oxy- or metmyoglobin. Against all expectations no analytical relation could be made between the R-value and the percentage of oxymyoglobin (R^2= 0.5). This was caused by the large biological variation in meat colour, the inaccuracy of the RGB measuring method and the method of Krzywicki.

All meat samples showed the same distinct relation in time; when the percentage of oxymyoglobin decreased, the percentage of metmyoglobin rises. Meat packaged in modified atmosphere and stored at 2°C did display this best (R^2= 0.92). Storage of the meat at 7°C did show an accelerated increase of the percentage of metmyoglobin and decrease in the percentage of oxymyoglobin.
MAP 7°C and air 7°C did show a decline in the total amount of myoglobin in time.

6 Recommendations

It is recommended to repeat the experiments with meat containing less fat (e.g. lean beef). Then deviation in the measurement of R-, G- and B- value will be less, because fat has a whitening effect. Also the isolation of the myoglobin will be easier.

Moreover, the method of Kubelka-Munk (Mancini *et al.*, 2003 and AMSA, 1991) is recommended. Meat is a natural product and each piece of meat is different in composition. The research described in this report needs a single piece of meat for each measurement, which means the starting point is never the same. When using the method of Kubelka-Munk, it is possible to use the same piece of meat for each measurement. The starting point is standardised and the deviation in the measurements is possibly less.
The method of Krzywicki, used in this research, is not suitable to quantify the percentage of deoxymyoglobin. The proposed method of Kubelka-Munk does not require a homogenisation step. Therefore there is less chance the conformation of deoxymyoglobin changes to oxymyoglobin. The method of Kubelka-Munk was not used in this research because of the use of hazardous chemicals.

In this research the RGB value of meat is calculated by averaging four different RGB measurements on a piece of meat. During this research it appeared it is possible to measure the RGB with a computer program designed at Agrotechnology & Food Innovations (personal communications 1). It was not yet possible to work with this program because of practical reasons. This program measures eight different colours of red, its surface and is able to ignore fat. Form the red colours and the surface of each colour it is possible to calculate the average R-, G-, and B-value of the whole piece of meat. This program might be more accurate because it takes a lot of measuring points and not only the four points that are used in this research. Besides measuring the RGB it is also recommended to measure the L*a*b* values. It is interesting to compare these results because McKenna (McKenna *et al.*, 2005) did show a relation between the a* value (redness) and the percentage of oxy- and metmyoglobin.

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Anonymous 2: http://bovine.unl.edu

Anonymous 3: http://www.vlees.nl

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Appendices

Appendix 1: The reactions taking place during the oxidation of oxymyoglobin to metmyoglobin.
(Gorelik and Kanner, 2001)

$Mb\text{–}Fe^{2+}\text{–}O_2 \leftrightarrow Mb\text{–}Fe^{2+}O_2$

$Mb\text{–}Fe^{2+} + O_2 \rightarrow Mb\text{–}Fe^{3+} + O_2^{\cdot -}$

$O_2^{\cdot -} + O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2$

$Mb\text{–}Fe^{3+} + H_2O_2 \rightarrow {}^{\cdot}Mb\text{–}Fe^{4+}{=}O + H_2O$

$Mb\text{–}Fe^{2+} + H_2O_2 \rightarrow Mb\text{–}Fe^{4+}{=}O + H_2O$

$Mb\text{–}Fe^{2+} + ROOH \rightarrow Mb\text{–}Fe^{3+} + RO^{\cdot} + HO^-$

$Mb\text{–}Fe^{2+} + RO^{\cdot} \rightarrow Mb\text{–}Fe^{3+} + RO^-$

$Mb\text{–}Fe^{2+}\text{–}O_2 + P\text{–}Fe^{4+}{=}O + H^+ \rightarrow 2Mb\text{–}Fe^{3+} + HO^- + O_2$

$Mb\text{–}Fe^{2+}\text{–}O_2 + Fe^{3+} \rightarrow Mb\text{–}Fe^{3+} + Fe^{2+} + O_2$

$Mb\text{–}Fe^{2+}\text{–}O_2 \Rightarrow$ oxymyoglobin
$Mb\text{–}Fe^{2+} \Rightarrow$ deoxymyoglobin
$Mb\text{–}Fe^{3+} \Rightarrow$ metmyoglobin
${}^{\cdot}Mb\text{–}Fe^{4+}{=}O \Rightarrow$ oxymyoglobin ferryl cation radical
$Mb\text{–}Fe^{4+}{=}O \Rightarrow$ myoglobin ferryl

Appendix 2: Nutritional value and the information belonging to the rib eye used in this research.

Nutritional value of rib eye.

Rib eye			
Value per 100 g raw meat			
Energy (kJ)	713	Vitamin B1 (milligram)	0.04
Energy (kCal)	171	Vitamin B6 (milligram)	0.09
Protein (gram)	20.8	Vitamin B12 (microgram)	2.2
Fat (gram)	9.7	Vitamin D (microgram)	0.4
Saturated fatty acids (gram)	4.4	Iron (milligram)	2.5
Unsaturated fatty acids (gram)	4.1	Zinc (milligram)	4.43
Cholesterol (milligram)	63.5	Sodium (milligram)	58

(Anonymous 3)

Information belonging to the rib eye used in this research.

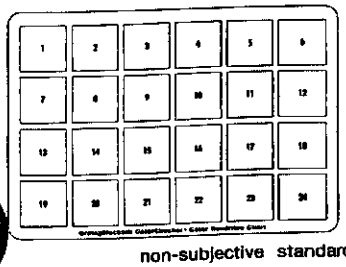
Born and bred in Hungary
Slaughtered in Hungary: HU-351
Carving in The Netherlands: EEG-29 NL
Reference number: HU 090505
Date of slaughter: 06-05-2005
Date of production: 09-05-2005
Shelf life (35 days): 10-06-2005

Appendix 3: Colorchecker® by Gretag Macbeth™, used for the calibration of the camera.

ColorChecker

Using the ColorChecker color rendition chart.

■ The ultimate goal of any process of photography, electronic publishing, printing, or television is to reproduce all colors perfectly. However, color rendition is a very subjective matter. Different renditions may be preferred for different applications, and different people may have varied color preferences.



■ To help make meaningful judgments about color rendition, a totally non-subjective standard of comparison is needed. That is why the GretagMacbeth ColorChecker chart was developed. It provides the needed standard with which to compare, measure, and analyze differences in color reproduction in various processes.

■ The ColorChecker is a checkerboard array of 24 scientifically prepared colored squares in a wide range of colors. Many of these squares represent natural objects of special interest, such as human skin, foliage, and blue sky. These squares are not only the same color as their counterparts, but also reflect light the same way in all parts of the visible spectrum. Because of this unique feature, the squares will match the colors of natural objects under any illumination and with any color reproduction process.

■ The ColorChecker chart provides an easy way to recognize and evaluate the many factors that can affect color reproduction. To evaluate the effect of varying any given factor, simply compare the chart's color image as it appears on the photograph, television picture, computer monitor, or printed sample — with the actual ColorChecker. This comparison may be made visually or through optical density measurements.

1.	dark skin	.400	.350	10.1	3 YR	3.7 / 3.2	moderate brown
2.	light skin	.377	.345	35.8	2.2 YR	6.47 / 4.1	light reddish brown
3.	blue sky	.247	.251	19.3	4.3 PB	4.95 / 5.5	moderate blue
4.	foliage	.337	.422	13.3	6.7 GY	4.2 / 4.1	moderate olive green
5.	blue flower	.265	.240	24.3	9.7 PB	5.47 / 6.7	light violet
6.	bluish green	.261	.343	43.1	2.5 BG	7 / 6	light bluish green
7.	orange	.506	.407	30.1	5 YR	6 / 11	strong orange
8.	purplish blue	.211	.175	12.0	7.5 PB	4 / 10.7	strong purplish blue
9.	moderate red	.453	.306	19.8	2.5 R	5 / 10	moderate red
10.	purple	.285	.202	6.6	5 P	3 / 7	deep purple
11.	yellow green	.380	.489	44.3	5 GY	7.1 / 9.1	strong yellow green
12.	orange yellow	.473	.438	43.1	10 YR	7 / 10.5	strong orange yellow
13.	blue	.187	.129	6.1	7.5 PB	2.9 / 12.7	vivid purplish blue
14.	green	.305	.478	23.4	0.25 G	5.4 / 8.65	strong yellowish green
15.	red	.539	.313	12.0	5 R	4 / 12	strong red
16.	yellow	.448	.470	59.1	5 Y	8 / 11.1	vivid yellow
17.	magenta	.364	.233	19.8	2.5 RP	5 / 12	strong reddish purple
18.	cyan	.196	.252	19.8	5 B	5 / 8	strong greenish blue
19.	white	.310	.316	90.0	N	9.5 /	white
20.	neutral 8	.310	.316	59.1	N	8 /	light gray
21.	neutral 6.5	.310	.316	36.2	N	6.5 /	light-medium gray
22.	neutral 5	.310	.316	19.8	N	5 /	medium gray
23.	neutral 3.5	.310	.316	9.0	N	3.5 /	dark gray
24.	black	.310	.316	3.1	N	2 /	black

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Hong Kong: 12A Wardley Centre, 9-11 Prat Avenue, Tsimshatsui, Kowloon, Hong Kong, Tel: +852 2368 7738, Fax: +852 2568 6717

The ColorChecker chart is produced in the Munsell® Color Lab at GretagMacbeth.

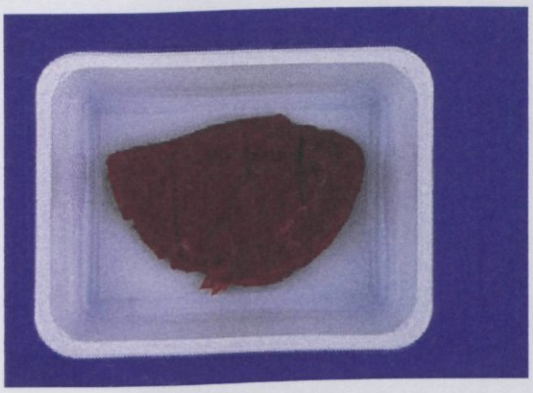
Appendix 4: Method to convert myoglobin in 100% deoxymyoglobin, oxymyoglobin or metmyoglobin.
(AMSA, 1991)

100% deoxymyoglobin
- place the meat in a 10% sodium dithionite solution for 1 minute
- drain and blot the surface of the meat
- vacuum package to reduce for 1 to 2 hours at room temperature

100% oxymyoglobin
- flush the meat in a closed cell with 100% oxygen for 10 minutes

100% metmyoglobin
- place the meat samples in a 1% potassium ferricyanide solution for 1 minute
- drain and blot the surface of the meat
- package in oxygen permeable film to oxidise at 2°C for 12 hours

100% deoxymyoglobin



100% oxymyoglobin

100% metmyoglobin

Appendix 5: CD with all the results, original data and digital photo's.