University of São Paulo
"Luiz de Queiroz" College of Agriculture

High-oxygen modified atmosphere package improves color stability of *Longissimus lumborum* with high ultimate pH from pasture-fed Nellore bulls

Caio César de Sousa Ribeiro

Dissertation presented to obtain the degree of Master in Science. Area: Food Science and Technology

Piracicaba
2017

Caio César de Sousa Ribeiro
Pharmacist-Biochemist

High-oxygen modified atmosphere package improves color stability of *Longissimus lumborum* with high ultimate pH from pasture-fed Nellore bulls

versão revisada de acordo com a resolução CoPGr 6018 de 2011

Advisor:
Profª. Drª. **CARMEN JOSEFINA CONTRERAS CASTILLO**

Dissertation presented to obtain the degree of Master in Science. Area: Food Science and Technology

Piracicaba
2017

Dados internacionais de Catalogação na Publicação

DIVISÃO DE BIBLIOTECA – DIBD/ESALQ/USP

Ribeiro, Caio César de Sousa

High-oxygen atmosphere package improves color stability of *Longissimus lumborum* with high ultimate pH from pasture-fed Nellore bulls / Caio César de Sousa Ribeiro - - versão revisada de acordo com a resolução CoPGr 6018 de 2011 - - Piracicaba, 2017.

52 p.

Dissertação (Mestrado) - - USP / Escola Superior de Agricultura “Luiz de Queiroz”.

1. Escurecimento de carne bovina 2. Tempo de exposição de carne à luz fluorescente 3. Atividade redutora da metamioglobina 4. Mioglobina 5. Oxidação lipídica I. Título

I dedicate this work to my family, friends, and professors for who have always supported me, even when I could not support myself

ACKNOWLEDGMENTS

Initially, I need to thank God for giving me so many opportunities, even if I do not recognize it.

This work is a fulfilled dream and was possible only because my family have supported me all the time. With or without financial condition, they have enabled I had condition to study. And to study only. During this time in Piracicaba, they faced my absence with love, patience, kindness, and comprehension unconditionally. And I am aware they will “care for me beyond measure and cherish me in their heart” forever. So, for what I am and for whoever I will become, I am thankful for my grandmother (*in memorian*), my father, my mother, and my brother. Of course, I have to be thankful for having a cute nephew, whose laughter floods my soul.

I thank to my university ESALQ for making this work possible and for the knowledge I had access during Master studies and to CAPES for funding my scholarship.

I thank Dr. Anna Cecilia Venturini from Federal University of São Paulo (UNIFESP, SP, Brazil) for having presented me this project and Meat Lab at ESALQ. She has always been willing to assist me, enabling then this research. So, it is fair to say she changed my professional life and for it I am grateful. Along with Dr. Anna, this work was able due to the Dr. Carmen J. Contreras-Castillo’s advisory. I thank my advisor for challenging me and for seeing the best of me, either as a person or as a professional. Obviously, two models of humble and qualified professors to look up to. The interpretation of color stability results’ was possible only because of the immense knowledge of Dr. Melvin C. Hunt from University of Kansas (USA) and Dr Ranjith Ramanathan from Oklahoma State University. Performing new analyses is challenging and it is pleasant to know you can count on two giants of Science.

I thank to professors Anna Venturini, Sergio Pflanzer, Eduardo Delgado, Roberto Roça, Saulo Silva, and Marco Trindade for having accepted this dissertation, adding greatly to the improvement of this work. Each of them contributed to this work. I thank especially to professor Saulo for proving material for color studies I used at MRA analysis.

I thank to the research group present when I started studying in 2014: Juan Sebastian, Mirian, Erick, Juan Dario, Beatriz, and Jair. I thank to Kathelyn who started this project and, on whose dissertation I based this study. And I thank to group that came after: Izabella, Thais, Carol, Mariana M, Mariana D, Bruna, Kamilla, Eugénie, Grazielle, Mariane, and Laura, This work was possible because of the hands and minds of special people: Felipe, Marcio, Carol, Thais, Giuliana, Bruna, and Kamilla. They helped me throughout the analyses and gave me a kind friendship I needed to bear the mishaps of research.

I have to say this work was produced because of the support, love and friendship I have received from my friends: Ruth, Mário, Filipe (CEFET), Anderson, Leu, Sérgio, Ana, Nathália, Elene, Gil, Giovana, Dan, Sakamoto, Doug, Joyce, Gisele, Barbara, Ronaldo (UNIFESP), Tyby, Daniel (USP-

SP), Dona Amábile, Carol, Thais, Giuliana, Gian, Bruna, Kamilla, Renata, Natália, Karina, Giovana, Thiago, Cesar, Patricia, Carlota, Regina, Rose (ESALQ), Rafael, Fagner, Lucas, Bruno, Adjailton, (my home in Piracicaba) Alais, Bruno, and Leda (FATEC Piracicaba). They made my research happier.

So, I am grateful for all the people who helped me over these three years and with whom I hope stay in touch for all my life.

EPIGRAPH

“It is our choice, Harry,

that show what we truly are,

far more than our abilities”

Albus Percival Wulfric Brian Dumbledore

(Joanne “JK” Rowling in Harry Potter and the Chamber of Secrets)

CONTENTS

RESUMO

Embalagem de atmosfera modificada com alto oxigênio melhora a estabilidade de cor de *Longissimus lumborum* com alto pH final de bovinos Nelore macho inteiro criado a pasto

A cor vermelho-brilhante da carne bovina é um atributo de qualidade essencial considerado no momento de compra pelo consumidor, sendo intrinsecamente afetada pelo pH final (pHf) da carne. Assim, esse trabalho objetivou determinar se o pHf alto da carne afetou a estabilidade de carne do músculo Longissimus lumborum (LL) de bovinos da raça Nelore macho inteiro criado a pasto e se a embalagem com atmosfera modificada com alta concentração de oxigênio melhorou a estabilidade de cor da carne com pHf alto. Para isso, 18 músculos *LL* de Nelore machos inteiros criados a pasto foram classificadas em 3 faixas de pHf: normal (n = 6; 5.40 < pHu <5.79), intermediário (n = 6; 5.80 < pHu <6.19) e alto (n = 6; pHu > 6.20), 48h pós abate. Todos os músculos foram então porcionados em bifes com 2,5 cm de espessura 72h pós abate, os quais foram embalados em atmosfera composta por 80 % O2/ 20% CO2 (v/v), sendo finalmente armazenados a 2 ± 1 ºC no escuro até o 5º dia de tempo de exposição. No 5º dia, as amostras iniciaram a exposição à luz fluorescente até o dia 14 do período. As análises de pH, composição gasosa das embalagens, cor instrumental, pigmentos superficiais, atividade redutora da metamioglobina (ARM), taxa de consume de oxigênio (TCO) e oxidação lipídica foram realizadas ao longo do tempo de exposição. Os bifes com pHf alto apresentaram cor mais escura (L*), vermelha (a*, a*/b*), com melhor tonalidade (hue), menor metamioglobina superficial e maior ARM ($p < 0.05$). A embalagem com alta concentração de oxigênio aumentou a proporção de oximioglobina (OMB) superficial ao longo do tempo de exposição ($p < 0.05$), evidenciando uma cor vermelho-brilhante no grupo alto. O grupo Intermediário se mostrou menos escuro o grupo alto e mais prolongada estabilidade de cor que o grupo normal ($p < 0.05$). Assim, considera-se que a pHf alto afetou a estabilidade de cor e de oxidação lipídica dos músculos e a coloração do grupo alto foi melhorada pela ação da embalagem com oxigênio. O pH Intermediário se mostrou vantajoso por apresentar benefícios presentes nos dois outros tratamentos.

Palavras-chave: Escurecimento de carne bovina; Tempo de exposição de carne à luz fluorescente; Atividade redutora da metamioglobina; Mioglobina; Oxidação lipídica.

ABSTRACT

High-oxygen modified atmosphere package improves color stability of Longissimus lumborum with high ultimate pH from pasture-fed Nellore bulls

Red bright color is an important quality attribute that influences beef purchasing and is affected by beef pH. Therefore, the aim of this study was to determine if high ultimate pH affected color stability of longissimus lumborum (LL) steaks from pasture-fed Nellore bulls and if HiOx atmosphere package improved the color stability of high pHu muscles. To achieve these objectives, 18 *LL* muscles from Nellore bulls were grouped into 3 pHu ranges: normal (n = 6; 5.40 < pHu <5.79), intermediate (n = 6; 5.80 < pHu <6.19) and high (n = 6; pHu > 6.20). All the muscles were cut into 2.5 cm steaks and packaged in 80% O2/ 20% CO2 (v/v) and then stored at 2 ± 1 ºC under dark conditions until day 5 and under fluorescent light until day 14 (the end of display-time). pH, gas composition, instrumental color, surface pigment, metmyoglobin reducing activity (MRA), oxygen consumption rate (OCR), and lipid oxidation were determined throughout display-time. High pHu steaks were darker (L*), redder (a*, a*/b*), with better tone (Hue), less metmyoglobin and higher MRA ($p < 0.05$) than normal pHu samples. HiOX MAP increased surface OMb during display time in pHu > 6.2 ($p < 0.05$), showing a bright-red color in high group. Intermediate group was less dark than high group and had longer color stability than normal group ($p < 0.05$). Therefore, High pHu had great color and lipid oxidation stability and desired color due to HiOx MAP under cold storage for 14 days and intermediate pHu had beneficial aspects presented in both other treatments.

Keywords: Dark-cutting beef; Beef display-time; Metmyoglobin reducing activity; Myoglobin; Lipid oxidation

1. INTRODUCTION

Brazil is one of the World's largest beef producer and exporter, having produced 9.3 million metric tons and exported 1.7 million carcass weight equivalent in 2016 (USDA, 2017). *Bos indicus* genetics, especially Nellore breed, has a great share in Brazilian cattle herd, which comprises approximately 215 million head (FERRAZ; FELÍCIO, 2010; IBGE, 2015). Male bovines are important to Brazilian meat production and represented 61.4% of the total cattle slaughtered in Brazil in 2016 (IBGE, 2017).

After animal slaughter and exsanguination, a muscle acidification drops pH from 7.4 to 5.4 – 5.8 after 24 or 48 h (ultimate pH, pH_u) (OUALI et al., 2006). The muscle may have pH_u higher than 5.8 due to innumerous pre-harvesting stress factors, which were reviewed by Dunne, Monahan and Moloney (2011) and Ponnampalam et al. (2017). Pasture finishing is one of these factors, compared to grain feeding, for providing less content of energy/mass. This feeding system contributes by diminishing the glycolytic potential leading, making animals more susceptible to glycogen-depletion in case of pre-slaughter stress, which results in higher pH_u and, consequently, darker muscles (DUNNE; MONAHAN; MOLONEY, 2011; PRIOLO; MICOL; AGABRIEL, 2001).

Because of being male and intact, bulls muscles also are related to increased beef pHu. Bulls have greater concentration of testosterone, which causes a more irritable behavior face stress factor, such as transport and human handling (SEIDEMAN et al., 1982; WĘGLARZ, 2010). This phenomenon makes bulls more susceptible to produce darker and less red muscles than those from castrated animals, as observed by Miguel et al. (2014) in pasture-fed purebred Nellore and 50% Nellore x 50% Aberdeen Angus compared to castrated and immunocastrated animals.

Darkening observed in high pHu beef concerns color researchers and meat processors, since color is the initial attribute that influences purchasing by consumers and loss of typical beef color – bright-cherry red – reduces consumer interest in beef (SUMAN et al., 2014).

Beef color is determined by two main factors: the concentration and state of myoglobin and the muscle micro-structure. Myoglobin assumes three major forms in fresh beef: deoxymyoglobin (DMb, purplish color, Fe^{2+}-H_2O), oxymyoglobin (OMb, bright-red color, Fe^{2+}-O_2) and metmyoglobin (MMb, brownish color, Fe^{3+}-H_2O). Oxygen depth and then the OMb layer depend on the temperature, partial pressure of oxygen, pH and the oxygen use by cellular components, such as mitochondria (MANCINI; HUNT, 2005). Air-exposition develops DMb blooming forming OMb on the muscle surface, which not occur properly in pH >5.8 (RENERRE, 1990). High pH also alters the muscle micro-structure due to reduction in light scattering by fibers getting swollen and then changing light penetration, which darkens the muscle (ABRIL et al., 2001; MACDOUGALL, 1982).

High pHu carcasses are reported to have greater mitochondrial oxygen consumption rate (OCR) due to a higher activity of the enzyme cytochrome c oxidase (complex IV), which oxidizes oxygen into water which results in less surface OMb and darker color (BENDALL; TAYLOR, 1972;

RENERRE, 1990; TANG et al., 2005). On the other hand, high pHu muscles have less myoglobin oxidation, resulting in a lower discoloration throughout display time due to accumulation of MMb on meat surface (RENERRE, 1990). This color stability also seems to be affected by pasture-finishing, since an increased OCR resulting from extensive management was noted by Dunne, Monahan and Moloney (2011).

Meat that is DFD is often relegated to use in manufactured meat products and is frozen prior to use (Ponnampalam et al., 2017). Packaging beef steaks in high-oxygen atmosphere (HiOx MAP), such as 80% O2/ 20% CO2, enhances beef bright-red color for increasing the surface OMb proportion and for retarding the uprising of MMb layer on the muscle surface (MANCINI; HUNT, 2005). Higher partial pressure of oxygen, found in HiOx MAP increases the depth of surface OMb, which can reduce darkening associated with high pHu beef (RENERRE, 1990). However, there is currently very limited information on any relationship with high pHu beef and lipid oxidation.

Color stability studies in pH > 5.8 muscles are published mostly from Bos taurus beef (ABRIL et al., 2001; ENGLISH et al., 2016; MCKEITH et al., 2016). There is a lack of researches investigating Nellore beef with increased pHu. Moreover, there is not investigation evaluating the improvement of darkening and lower redness found in high pHu with packaging in HiOx. Therefore, this study aimed to determine if high ultimate pH affected color and lipid oxidative stability of longissimus lumborum (LL) steak from pasture-fed Nellore bulls and if HiOx atmosphere package increased color saturation and redness in high pH_u steaks.

2. REVIEW OF LITERATURE

2.1. Meat color and myoglobin chemistry

2.1.1. Myoglobin chemistry

Although many factors influence meat color, such as muscle micro-structure, myoglobin is the major responsible for color characterization and changing in meat, especially from livestock species. Binding to environment molecules, content and oxidation are some of causes of color altering in meat.

Myoglobin is a sarcoplasmic heme protein present in muscle cells, which acts as a short term oxygen (O_2) reserve in live organism (GRABER; WOODWORTH, 1986). Posterior to animal slaughter and exsanguination, myoglobin is the major pigment that results in the red color of meat, especially in livestock species. The concentration of myoglobin and its redox state are two constitutional factors that determine meat color (MANCINI; HUNT, 2005).

Structurally, myoglobin is composed of a polypeptide chain and a group of ferric protoporphyrin, called heme. Heme consists of tetrapyrrolic ring with conjugated double bonds, which binds coordinately a ferric iron. The resonant electronic distribution from these conjugated bonds can absorb visible light, contributing to myoglobin showing color. Heminic iron forms six coordinate bonds. Four of these bonds are with heme pyrrole nitrogens on the same plane. Fifth coordination involves interaction between iron and proximal histidine, located in the position 93 (His93). Sixth site of heme is available for reversibly binding with a small ligand, such as O_2, nitric oxide (NO) and carbon monoxide (CO). Fifth and sixth bonds are perpendicular to the heme ring. Heminic iron may bind to O_2 as result of myoglobin's hydrophobic pocket, which is proper to heme location. Inside this pocket, accessibility of heme group to solvent is highly restrict, which protects iron from oxidation. Bound to iron, O_2 forms hydrogen binding to distal histidine (His64), which improves its binding.

Since myoglobin provides red color, its concentration determines the muscle darkening. Genotype, castration, physical exercise, age, and the muscle type are some of the many factors that affect myoglobin concentration in the muscle (SUMAN et al., 2014). Researching seven breeds (Angus, Charolais, Gelbvie, Hereford, Limousin, Red Angus, and Simmental), King et al. (2010) demonstrated the effect of genotypes on myoglobin content, having Simmental the greatest concentration (3.71mg/mL), whereas Charolais and Limousin had significantly less (2.77 and 2.72 mg/mL, respectively). Devol et al. (1985) reported intact males (bulls) had more meat pigment than steers (3.25 vs 2.9 mg/g tissue, respectively). Due to long-term physical exercise and then to necessity to store O_2 during walking, pasture-raised cattle show a more content of myoglobin in the muscle than feedlot-finished (DUNNE; MONAHAN; MOLONEY, 2011). Since older animal's myoglobin has less affinity to O_2, a greater content of myoglobin is needed to store O_2, which increases myoglobin concentration and, then, darkens the meat.

Red fibers have higher myoglobin concentration and capillary blood flow than white ones, which maintains O_2 supply to cells. These two characteristics allow red fiber to oxidize glycose via aerobic pathways, which is corroborated by the larger number of mitochondria and tricarboxylic acid enzymes in muscle cells (CHOI; KIM, 2009). The muscle structure also contributes to color developments for affecting O_2 influx through the tissue, enabling interaction between gas and myoglobin (MANCINI; HUNT, 2005).

In fresh meat, myoglobin assumes 3 forms: deoxymyoglobin (DMb), oxymyoglobin (OMb), and metmyoglobin (MMb). Beef contains these three chemical forms simultaneously under natural conditions, such as at retail store or at home, and they occur in dynamic equilibrium, as shown in Fig. 1 (AMSA, 2012). The higher proportion of one pigment is dependent on intrinsic and environmental factors, such as meat pH_u, meat reducing activity, lipid oxidation, gas composition, temperature, and microbial growth (MANCINI; HUNT, 2005).

Chronologically, at the moment of slaughter myoglobin does not have ligand at the sixth coordination site of Fe^{2+}, assuming DMb form and, then, conferring purplish-red or purplish-pink color to muscle. After slaughter, myoglobin is exposed to O_2 from air, being oxygenated, with OMb form and bright-red color. This meat can be discolored during retail time or at home by oxidation of myoglobin ferrous iron to ferric form, generating MMb, conferring brownish color to the meat (BEKHIT; FAUSTMAN, 2005; MANCINI; HUNT, 2005).

During oxygenation, also called blooming, O_2 binds to the sixth coordination site of myoglobin iron. This blooming takes place more efficiently when meat is exposed to air at temperature near to 0-2 ºC for, at least, 30 minutes. Higher temperature increases O_2 consumption (OC) by cellular mitochondria, and these organelles are competitor to myoglobin oxygenation (BENDALL; TAYLOR, 1972; RENERRE, 1990). A thicker OMb layer can be formed on the steak by storing it in high O_2 modified atmosphere package (HiOx MAP).

Nowadays, vacuum-packaged meat is getting more common to be purchased because of the practicality to processors and consumers. However, vacuum-packaged muscles appear dark and purplish, due to the DMb layer on the surface, which can be easily oxygenated in the air. A high concentration of DMb is also observed in muscle immediately after cutting, since O_2 penetration does not occur through the entire meat (MANCINI; HUNT, 2005). The absence of OMb on a steak immediately after cutting is particularly useful for oxygen consume rate analysis to measure oxygen intake by mitochondria, which will be reviewed in section 2.5.

Contrary to OMb and DMb, MMb represents losses to meat market, due to its brownish color, which is interpreted as loss of quality. MMb generation begins beneath surface, between superficial OMb and inner DMb layers. In the intermediate layer, O_2 tension cannot oxygenate all available DMb, which can react with reactive oxygen species (ROS) product of mitochondrial metabolism (O'KEEFFE; HOOD, 1982). During heme oxidation, the subsurface layer of MMb

increases and moves towards the surface, as surface OMb layer becomes thinner and are replaced by MMb layer, browning meat surface (MANCINI; HUNT, 2005).

The conversion of OMb to MMb is thermodynamically unfavorable. Oxidation, then, requires the deoxygenation to DMb from OMb, which occurs naturally in dynamic disassociation equilibrium. DMb formed, in contact with ROS can rapidly be oxidized to MMb. However, some catalyzers can turn oxidation more favorable in aerobic conditions, such as free metal ions (iron and cooper), for reacting with O_2 and generating ROS, as shown by George and Stratmann (1952). Besides low tension of O_2, low pH_u, high temperature, lipid oxidation and loss of MMb-reducing activity are factors that influence iron oxidation (BEKHIT; FAUSTMAN, 2005; MANCINI; HUNT, 2005).

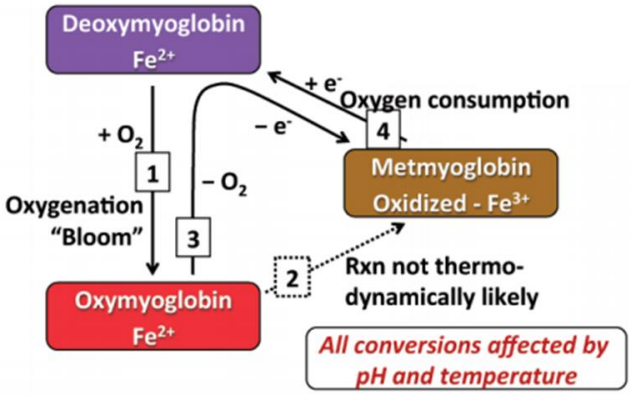

Fig 1. Interconversions of three chemical forms of myoglobin in fresh beef (AMSA, 2012).

2.1.2. Color stability

Fresh beef contains all three myoglobin forms DMb, OMb and MMb simultaneously under natural conditions and they are in dynamic equilibrium. Due to biochemical changes, this chemical equilibrium can be displaced during beef shelf-life, favoring MMb formation by myoglobin oxidation (BEKHIT; FAUSTMAN, 2005), which compromises beef color stability. Along with MMb formation, beef can loss quality related to color due to the reduced oxygenation of myoglobin, which strengthen the OMb layer (MANCINI; HUNT, 2005). Reduced oxygenation and oxidation of myoglobin are regulated by mitochondrial enzymes (RENERRE, 1990; TANG et al., 2005). Both factors affect beef color stability, entailing on financial and food quality losses (SMITH al., 2000), given that consumers tend to reject dark muscles, with high pH_u, as reported by Viljoen, de Kock and Webb (2002).

The increase of MMb proportion throughout retail time involves decreasing of efficiency of MMb reducing activity (MRA) (BEKHIT; FAUSTMAN, 2005). MRA is an intrinsic cellular ability to reduce MMb to produce DMb, which can be oxygenated to OMb. MRA seems to be critically important to maintain surface color stability and the aspects influencing it require deeper consideration in color stability studies.

MMb reduction can be carried out by enzymatic or non-enzymatic systems. The enzymatic system is considered the most important for reconverting MMb to DMb. This mechanism involves the enzyme NADH-cytochrome b5 MMb reductase and bases on the transference of two electrons from a coenzyme NADH to a ferricytochrome b5, reducing it to ferrocytochrome b5. As an intermediate, ferricytochrome b5 non-enzymatically reduces MMb to DMb, regenerating the content of oxidized cytochrome b5 and improving meat color (ARIHARA et al., 1995; BEKHIT; FAUSTMAN, 2005). Furthermore, MMb also may be reduced via electrons from mitochondrial electron-transport chain to form DMb (BELSKIE et al., 2015; TANG et al., 2005).

As retail time continues, MMb reductase activity and NADH reduces gradually, influencing color stability (MANCINI; HUNT, 2005). Kim et al. (2009) observed a pattern for the decrease of LDH-B activity, NADH concentration and the percentage of MRA during retail time of longissimus lumborum (LL) muscle packaged under fluorescent light for 7 days with a PVC film covering.

Few MRA researches have been conducted with Nellore breed (*Bos indicus*), widely raised and slaughtered in tropical countries, such as Brazil and India. Canto et al., (2016) evaluated MRA in Nellore longissimus lumborum (*LL)* and *psoas major* (*P. major*) packaged on polystyrene trays with O_2-permeable film and stored refrigerated for 9 days. The authors observed a progressive falling during all retail time for the both muscles. Likewise Canto et al. (2016), other authors have reported the inverse relation between MRA and meat discoloration (BEKHIT; FAUSTMAN, 2005; LEDWARD, 1985; MANCINI; SEYFERT; HUNT, 2008; REDDY; CARPENTER, 1991; SEYFERT et al., 2006). Longissimus showed more MRA than *P. major* (PM) on each day, which is expected due to its color stability compared to PM. The role of enzyme MMb reductase appears as determinant of meat color stability, indicating the higher enzymatic activity, the more stable is the color presented by the muscle (REDDY; CARPENTER, 1991). This higher activity, associated with higher MRA, may explain the greater color stability of some muscles, such as *LL*, compared to *P. major*, as observed by Canto et al., (2016) and Madhavi and Carpenter (1993).

However, the relation between MRA and discoloration is not consensual. Several investigators have not observed improving of surface color as MRA progresses (ECHEVARNE; RENERRE; LABAS, 1990; O'KEEFFE; HOOD, 1982). Although Kim et al. (2009b) have found MRA decreased, the authors did not find increasing in surface MMb accumulation (%) in beef *LL* steaks stored for 7 days at 1 ºC. According to Sammel et al. (2002), the lack of uniformity in the MRA methodologies could explain these contradictory findings.

Another factor that influences color stability is the mitochondrial OC, as observed by numerous authors (BENDALL; TAYLOR, 1972; MADHAVI; CARPENTER, 1993; RAMANATHAN et al., 2013; SEYFERT et al., 2006; TANG et al., 2005). Oxygen consumption rate (OCR) has been reported to stabilize color in synergism with MRA (SAMMEL et al., 2002; TANG et al., 2005). As a consequence of the oxygen consumption by mitochondria, a low partial pressure of O_2 and ROS are generated, which favor myoglobin oxidation (TANG et al., 2005).

Cytochrome c oxidase (complex IV) has been reported to be essential to OCR (GRABEŽ et al., 2015; SEYFERT et al., 2006). This enzyme is located on the inner mitochondrial membrane and comprises the electron-transport chain along with complexes I (NADH oxidoreductase), II (succinate-Q reductase), III (coenzyme Q-cytochrome c oxidoreductase). During mitochondrial respiration, cytochrome c oxidase catalyzes the formation of water by the O_2 diffused through meat, proton and electrons. Mitochondrial OCR decreases the O_2 concentration available to interact to myoglobin to form OMb. This competition determines the depth of O_2 penetration into exposed muscle, affecting the surficial bright-red color (MANCINI; HUNT, 2005). Thus, a muscle with high OCR tends to present darker than its counterpart with low OCR.

Meat OCR is reduced during postmortem aging. Over the time, there is improvement in color development because of decrease in OC. This phenomenon direct or indirectly was observed by Lindahl (2011) for longissimus and semimembranosus muscles; Tang et al. (2005) for bovine cardiac muscle on 2h, 6h and 60h; and (Mancini and Ramanathan (2014) for *LL* on 0, 15, 30, and 45d of chilled aging. Depletion of the substrates for OC, such as lactate, succinate, and NADH, during storage time makes O_2 diffuse more rapidly through tissue to bind to myoglobin, which improves color development and stability (MACDOUGALL, 1982; MANCINI; RAMANATHAN, 2014).

2.2. Beef ultimate pH

Ultimate pH (pH_u) is the pH value the meat present after stabilization of the pH drop, posterior animal slaughter. Although some researchers measure pH_u after 24 h postmortem (GALLO, 2004; PULFORD et al., 2008), pH_u seems to stabilize after 48 h postmortem. A decline in pH_u values is observed when this is measured after 24 and 48 h postmortem. Moreover, the not-completely stable drop profile is shown by Ouali et al. (2006) and observed by (Mlynek, Janiuk, and Dzido, (2012).

Ultimate pH higher than 5.8 is an issue present in many regions worldwide. Mach et al. (2008) reported incidence of 13.9% to pH>5.8, 24 h postmortem in Spain, whereas Gallo (2004) reported data from abattoirs from Brazil's Southern region, with incidence from 17 to 40% annually. According to Contreras-Barón (personal communication), most of bovines slaughtered in Brazil have shown pH above 5.8, 24 h postmortem, which may impair Brazilian exportation. Nevertheless, there are not records yet of Brazilian slaughter system on incidence of abnormal pH in meat.

2.2.1. Biochemical basis of ultimate pH

The live muscle has neutral pH (7.2-7.4). Posterior to animal exsanguination during slaughter, the delivery of O_2 to muscle cells is ceased. Without O_2, cells cannot metabolize glycose aerobically, by tricarboxylic acid pathway and respiratory chain. Glycose generated from muscle glycogen must be then metabolized by glycolysis to pyruvate and this is converted to lactate and ions H^+, whose accumulation acidifies the muscle cells (ENGLAND et al., 2016; SCHEFFLER et al., 2015).

The pH_u of normal beef *LL* is approximately 5.4-5.79 (LOMIWES et al., 2014). In glycolytic muscles, the reduction of pH is inversely correlated to initial concentration of glycogen in the muscle and glycolysis occurs until achieving normal pH_u, in normal conditions (Lawrence; Fowler; Novakofski 2012). Achieved acid pH, a regulatory enzyme, phosphofructokinase, is then inhibited, ceasing glycolysis and the pH decline (ENGLAND et al., 2014).

The rate of pH falling depends on the rate of glycolysis, the time necessary to reaching pH_u and to entering rigor mortis and varies from each muscle (Lawrence; Fowler; Novakofski 2012). Ultimate pH may vary according to the type of fiber that comprises a muscle. As stated by Talmant et al. (1986), the rate of acidification can be faster in muscles with more proportion of glycolytic fibers than in ones with lesser, as they have greater content of glycogen than oxidative fibers.

This pH decline until 5.4-5.79 is not a continuous curve. The fall profile of pH presents transient pH stability steps, as observed in two 19-month-old Charolais bulls' *Longissimus* muscle; during 0-10 hours post-slaughter (OUALI et al., 2006). Authors associated these pH drop profile with phospholipid-dependent inversion of polarity in cellular membranes. In this phenomenon, the electronegative phosphatidylserine groups switch to the external leaflet of membrane, while electropositive phosphatidylcholine and phosphatidylethanolamine groups are changed to internal leaflet, when apoptosis happens in muscle cells. This switching causes transient partial neutralizations of protons formed from glycose by glycolysis (OUALI et al., 2006).

Muscle glycolytic potential is the molar sum of all substrates to glycolysis (glycogen, glucose, glucose-6-phosphate, and lactate). Many researchers have demonstrated the negative correlation between glycolytic potential and ultimate pH (HOLDSTOCK et al., 2014; MCKEITH et al., 2016; WULF et al., 2002).

2.2.2. Factors influencing ultimate pH

Meat researchers have been reporting that the normal pH_u for beef is 5.4 – 5.8. Achieving normal pH_u range can be hampered by many pre-harvest factors, such as lack of castration, the type of finishing, physical exercise, nutrition, and pre-slaughter stress, such as lack of familiarity with being handled, exercise, fights and animal abuse by humans (DUNNE; MONAHAN; MOLONEY, 2011; PONNAMPALAM et al., 2017).

Intact bovines have been classically reported for resulting in high pH_u meats, as reviewed by (Seideman et al. (1982) and reported by Węglarz (2010). The excitable temperament, showing aggressive and sexual activities, presented in bulls is linked to testosterone (SEIDEMAN et al., 1982), which is related to glycogen depletion before slaughter, hindering the proper muscle acidification. On contrary, many researchers have been showing that different temperament categories did not resulted in different pH_u values (for example KING et al., 2006).

A higher pH_u and lower glycolytic potential have been attributable to pasture-raised beef cattle and submitted to long-term physical exercise (DUFRASNE et al., 1995; VESTERGAARD et al., 2000; YOUNG et al., 1997). Although long-term exercise promotes increase in myoglobin content in the muscle, observed by Priolo, Micol and Agabriel (2001), it is not shown by DUFRASNE et al., (1995). Pre-slaughter stress during transport and handling by a strange human presence and the high-energy diet represented by grains are some reasons which base his behavior (IMMONEN et al., 2000). In contrast, Dunne et al. (2005) did not find difference between 4.41 km walk exercised 18-month-old steers and control group regarding 48 h pH_u.

A special factor that happens worldwide and that influences pH drop profile is the animal's undernutrition (BRAY; GRAAFHUIS; CHRYSTALL, 1989; KANDEEPAN et al., 2009). As animals do not receive enough nutrient content to store glycogen in their muscles, less lactic acid is produced during rigor mortis process, which leads to a high pH_u meat.

Several factors provoke long-term or acute stress before slaughter, such as bull's irritable behavior and lack of animal welfare. Those elements deplete muscle content of glycogen, resulting in high pH_u meat (PULFORD et al., 2008). Peña and other (2014) reported there was sound stress in Charolais, Limousin and Retinta bulls after maintenance with pigs in the lairage prior to slaughter. There was a slightly but significant increase in pH_u in meat from the stressed group. The conditions of transportation (time, welfare, habit, bracing) contribute to stress animal before slaughter, as reviewed extensively by Ferguson and Warner (2008). These external influences on meat quality, observed in the meat pH_u, reinforce the necessity of the application of animal welfare, besides the ethical aspects.

2.2.3. Ultimate pH ranges

Meat pH_u ranges from 5.3 to over 7.0, as reported by several authors (ABRIL et al., 2001; CONTRERAS-CASTILLO et al., 2016; HUNT; HEDRICK, 1977; LAWRIE, 1958; MCKEITH et al., 2016; PULFORD et al., 2008). Nevertheless, grouping pH_u into ranges is not consensus, which results in several classifications, according to each research group.

Regarding beef color and tenderness studies, ultimate pH values ≤ 5.8 has been considered as normal to *Longissimus* muscle, whereas readings higher than 5.8 has been associated to high pH_u (PARK; LEE; HWANG, 2007; VILJOEN; DE KOCK; WEBB, 2002). In order to study meat tenderness,

some authors, such as Lomiwes et al. (2014) and Wu et al. (2014) grouped pH_u into 3 ranges: normal or low (≤ 5.79) intermediate (5.80-6.19) and high (≥6.20). It is can be attributable to the 3 groups observed for meat tenderness, being the intermediate value tougher than others (LOMIWES et al., 2013; PULFORD et al., 2008). However, these ranges employed in tenderness researches may not be applied in meat color studies. Along with pH equals 5.8, the pH_u value of 6.0 have been used as a threshold to classify pH_u for some authors, who grouped into normal or low pH_u when $pH_u \leq 6.0$ and high to $pH_u \geq 6.01$ (HOLDSTOCK et al., 2014).

Concerning $pH_u \geq 5.8$ for color studies, pH_u values may be or not grouped into further classes. Viljoen, Kock and Webb (2002) and Park et al. (2007) considered $pH_u \geq 5.8$ as a group to be studied. On the other hand, McKeith et al. (2016) sorted their high pH_u carcasses into 4 classes for *longissimus thoracis* muscles, according to dark color the carcasses showed: shady (pHu: 6.1 ± 0.03), mild (6.4 ± 0.03), moderate (6.6 ± 0.03), and severe (6.9 ± 0.03).

Since there is not a standard to grouping pH_u values in meat color researches, the cluster analysis used by Abril et al. (2001) seems to result in more reliable classification. The authors studied color evolution as a function of a wide number of pH_u readings and employed cluster analysis in order to generate the number of pH, according to the color data behavior. Thus, the number of ranges and the pH values used as threshold are dependent on the results' behavior.

Using the statistical package SPSS, Abril et al. (2001) employed a discriminant analysis with the selecting λ_{Wilks} parameter in order to obtain the most significant variables. By this discrimination, the authors determined that their previous pH_u ranges chosen for the study ($pH \leq 6.0$; $6.0<pH<6.5$; $pH \geq 6.5$) resulted in discrimination of approximately 60%, which was not appreciable by the authors. Cluster analysis, then, showed the X, Y, Z tristimulus values and the CIE L*, a*, b* co-ordinates results behaved as two groups: $pH < 6.1$ and $pH \geq 6.1$, which was used to reclassified the samples.

2.3. The effect of pH_u on meat color

Historically, many researchers have reported that pH > 5.80 results in darker meat (ABRIL et al., 2001; ASHMORE et al., 1971; HOLDSTOCK et al., 2014; HUNT; HEDRICK, 1977; PAGE; WULF; SCHWOTZER, 2001; STACKHOUSE et al., 2016), with incidence worldwide. Moore et al. (2012) reported 3.5% of the carcasses evaluated in the 2011 United States' National Beef Quality Audit. In Canada, Beef Cattle Research Council – BCRC (BCRC, 2013) reported 1.3% of incidence in 2010/2011. As regards Brazilian incidence of pH_u higher than 5.8, Rosa et al. (2016) studied 485 F1 crossbred bovines (Nellore x South African Simmental). From researched animals, 4.53% (n=22) presented pH > 5.8 and meat color was darker than normal pH_u. Although the percentage has not been great, given the volume of beef carcasses commercialized worldwide, a reduced percentage of the occurrence of undesirable color represents great losses for meat industry, as stated by Hughes, Kearney, and Warner (2014).

A typical denomination for beef darkening is dark-cutting beef (DC or DCB). Some authors have also reported it as DFD beef (Dark, Firm and Dry). But it is important to note that not every high pH_u muscles are tough. Due to enzymatic action by calpain proteases, high pH_u (especially above 6.3) can be as tender as normal pH_u (LOMIWES et al., 2013). Therefore, “dark-cutting” term seems more adequate for color studies.

As reported by Page et al. (2001), DC is greatly correlated with pH_u ($r = 0.80$). Authors showed 91.7% of the muscles with $pH_u \geq 5.87$ were classified as dark cutters, following USDA grading standards, whereas across normal pH_u muscles, the proportion was of 0.6%. Nonetheless, an atypical darkening may occur in normal pH_u muscles. Taking as pH_u cut-off of 6.0, Holdstock et al. (2014) reported 3 groups of LT muscles: a) Canada AA carcass muscles (Japanese Meat Grading Association – JMGA- score of 3.50 and pH on muscle of 5.57, n = 10); b) Canada B4 carcass muscles, being 1) atypical dark cutters (JMGA of 6.80 and pH on muscle of 5.83, n = 10), and 2) typical dark cutters (JMGA of 7.75 and pH on muscle of 6.62, n = 10). JMGA ranges from 1 (lightest color) to 8 (dark cutting). Both AA muscles and atypical dark cutter presented high glucidic potential (73.20 and 64.21 $\mu mol\ g^{-1}$ fresh tissue, respectively), which enables a proper pH falling to normal pH_u.

Increased pH_u provokes changing in many color parameters. Table 1 summarizes researches evaluating the effect of high pH_u on color attributes (instrumental, visual and color stability). The most used colorimetric system to characterize color or to evaluate color changings is the Commission Internationale de l’Eclairage (CIE) L*a*b*, where L* measures lightness (0: black, 100: white), a* measure redness (-60: green, +60: red), and b* measure yellowness (-60: blue, +60 yellow). Other systems are HunterLab, such as RGB (red, green and blue) model.

At a higher muscle pH_u, lightness (L*) diminishes due to a decrease in light scattering inside the structure of fiber (HUGHES et al., 2014; MACDOUGALL, 1982), since water is bound with protein charged groups and, hence, the muscle fibers are swollen. The high water-holding capacity also hinders O_2 penetration in meat, inhibiting OMb formation, which provides a bright-red color to meat. Furthermore, redness (a*) is reduced at a higher pH_u due to a decreased accumulation of OMb on the muscle surface because of a more intense activity of mitochondrial cytochrome c oxidase, during cellular respiration (BENDALL; TAYLOR, 1972; TANG et al., 2005). The increased mitochondrial activity in high pH_u also promotes an intense oxidation of molecular O_2 to produce water in electron-transport chain. According to Bendall and Taylor (1972), this reaction is approximately 50-75% higher at pH 7.2 than at pH 5.8. As a result, conversion to DMb redox form is favored in high pH_u, providing a purplish color to meat.

Despite high pH_u hampers the proper formation of OMb, an advantage of this pH_u range is the inhibition of myoglobin oxidation, since the rate of formation of MMb is pH dependent. Many researchers investigated the role of pH on the rate of MMb formation. According to George and Stratmann (1954), the rate of MMb formation in air is twice less favorable with an increase in pH from 5.4 to 6.6. Echevarne, Renerre, and Labas (1990) showed that MMb reductase activity increases in function to medium pH, achieving its maximum activity at pH 7.3. Ramanathan et al. (2012) reported

that at pH 7.4 a mitochondrial NADH-dependent reductase reduced significantly more MMb in control group than at pH 5.6. Therefore, steaks with higher pH_u are less susceptible to myoglobin oxidation.

This phenomenon is observed in hue results. Page et al. (2001) found that muscle pH_u was most correlated with a* and b* than with L*. The authors stated that pH had affected muscle color by changing hue. Reduction in hue values represents increment in color stability, with lower discoloration due to less MMb formation, which occurs at high pH_u (ECHEVARNE; RENERRE; LABAS, 1990). Other authors showed decreased hue and Chroma as pH_u increased and (ABRIL et al., 2001; MCKEITH et al., 2016; STACKHOUSE et al., 2016; WĘGLARZ, 2010). Lower Chroma indicates that as pH_u increases, surface color presents less vivid, losing normal pH_u bright red color, which may be attributable to higher OCR.

Table 1. Summary of publications on the effects of high ultimate pH on meat color researches

Experimental material	High pH_u	Findings	Publication
LT from Bos taurus (Parda Alpina (15) and Pirenaica (16) breeds)	> 6.1	CIE L*, a*, b*, Chroma, and hue were affected by pHu ($p \leq 0.001$). CIE b* is the best discriminant for pH_u groups.pH_u also had effect on reflectance spectra during ageing (0 min, 5h, 2d, and 9d). There was dependence between pH_u and the spectrophotometric indexes (K/S614 - K/S632, K/S 630 – K/S 580, and R∞632 - R∞614) ($p \leq 0.001$).	(ABRIL et al., 2001).
LL from steers (680), heifers (315) and bullocks (5).	≥ 5.87	Beef *LL* from 24/1000 carcasses were grouped into > 5.87 pH_u, being 22/24 carcasses classified as “dark cutters”. Muscle pH_u had positive correlation with DC (r=0.80) and negative correlation with L* (r=-0.40), a* (r=-0.58), and b* (r=-0.56).	(PAGE; WULF; SCHWOTZER, 2001).
LD from beef carcasses	≥ 5.80	High pH_u samples had lower sensory scores related to color than normal pH_u ($p \leq 0.001$)	(VILJOEN; DE KOCK; WEBB, 2002).
LT from Beef carcasses (47)	av. 6.06	High pH carcasses presented LT with lower values of CIE L*, a*, and b* than normal muscles ($p \leq 0.05$). DFD *LL* and MST 7 day post-mortem ($pH_u > 5.80$) also showed lower CIE L*, a*, and b* than normal muscles ($p \leq 0.05$).	(WULF et al., 2002)
LL from Hanwoo steers and bulls (24)	> 5.80	Correlation between beef darkness (lean meat color) and pH_u (r = 0.77). By CIE a*, normal pH_u muscles showed redder than high pH_u group.	(PARK; LEE; HWANG, 2007).
LT from young bulls (106), bulls (96), cows (317), and heifers (95)	≥ 5.80	pH negatively correlated with L* (r = -0.24), a* (r = -0.29), b* (r = -0.24), Chroma (r = -0.29), and hue (r = -0.30).	(WĘGLARZ, 2010).
MS from bulls (36) and heifers (24). 13-24 months-old.	≥ 5.80	Lower L*, a* (CIEL*a*b), R, G, B (RGB), V, and L values for high pH_u than for normal muscles ($p \leq 0.05$). Total heme pigment content did not increased in high pH_u group. pH showed correlation with L* (r=-0.80), R (r=-0.79), G (r=-0.69), B (r=-0.68), V (r=-0.79), and L (r=-0.77), and moderate with a* (r=-0.44).	(CHMIEL et al., 2012).
LT from 179 dark-cutting beef carcasses	> 6.0	Muscles with $pH_u > 6.0$ showed darker than normal group, based on JMGA ($p < 0.05$). Atypical DC muscles ($pH_u < 6.0$) also showed dark than normal group. Correlation (R^2) between pH_u values and JMGA scores of 0.59.	(HOLDSTOCK et al., 2014).
LL from beef carcasses (10) aged during 62 d under	av. 6.4 (all	High pH_u showed higher OCR and MRA, and lower CIE L* and surface OMb (during oxygenation	(ENGLISH et

vacuum (dark at 2 ºC)	aging)	period) for all aging time.	al., 2016).
LL from steers and heifers. 9-30 months-old	av. 6.1/ 6.4/ 6.6/ 6.9	Animal selection based on DC, with subsequent grouping into 4 sub-groups from average pH_u 6.1 to 6.9. Instrumental color (L*, a*, b*, Chroma, and hue) and color stability parameters (IMF and bloomed OMb) decreased as pH_u increased ($p \leq 0.05$).	(MCKEITH et al., 2016).
LL from beef carcasses (9)	av. 6.65	Non-enhanced DC muscles showed higher pH_u than normal pH_u group (USDA choice). High pH_u group (non-enhanced) were darker (L* and visual color), less red and with lower hue and Chroma values than non-enhanced normal pH_u muscles on initial retail time. Color parameters were more stable during retail time for high pH_u than for normal pH_u group.	(STACKHOUSE et al., 2016).

Muscles: *LL*: longissimus lumborum, LT: longissimus thoracis, LD: longissimus dorsi, LTD: latissimus dorsi, RA: rectus abdominis, MSM: semimembranosus, MST: semitendinosus. DFD: dry, firm, and dark meat, RGB: computer vision system (R: red, G: green, B: blue), CIE L*(lightness), a* (redness), b* (yellowness), av.: average.

2.4. HiOx MAP improving beef color

In order to increase the bright-red color on beef surface in high pH_u meat due to an increase in OMb proportion, storing and retailing steaks in high-oxygen modified atmosphere packages (HiOx MAP) could be useful. But, to achieve this extension, good hygiene and temperature control (cooling conditions) must be practiced during the whole beef supply chain until consumption (SUN; HOLLEY, 2012). Bendall and Taylor showed OCR is accelerated by temperature increase. Therefore, to obtain bright-red color due to surface OMb, trays should be retailed at low temperature, such as 1 ºC.

Some retail marketplaces have been packaging steaks and ground beef in modified-atmosphere packages. According to Brody (2007), approximately 80% of fresh and processed meat are found in MAP or vacuum-packages in US and UE. Among some benefits brought by using MAP – reduction of beef metabolism and lipid oxidation, spoilage control, and logistic benefits –, the bright-red color preservation during display-life is the most aimed (MANCINI; HUNT, 2005). MAP is a technology based on removing air from a package and replacing it with a gas or a mixture of gases, for achieving a predefined objective. The most employed gas and blend are nitrogen (N2), carbon dioxide (CO_2), O_2/CO_2, and, with restrict use, monoxide oxide (CO) (SUMAN et al., 2014).

HiOx MAP, higher than 50% O_2 is one of the most used blends of gases for beef steaks (JAYASINGH et al., 2002; MANCINI; HUNT, 2005; SUMAN et al., 2014). HiOx MAP enhances beef bright-red color for increasing the surface OMb proportion and for retarding the uprising of MMb layer on the muscle surface (MANCINI; HUNT, 2005). Whereas steaks packaged with O_2-permeable films can maintain the cherry-red color for hours or a few days, with HiOx MAP, beef can be displayed for 6-10 days (MCMILLIN, 2008), but not over 16 days, due to microbial spoilage (SUN; HOLLEY, 2012).

As regards microbial quality, HiOx MAP is useful to inhibit the growth of anaerobic microorganisms, such as lactic bacteria (POTHAKOS et al., 2015) in exposed-to-MAP surface. However, aerobic spoilage is favored to develop in aerobic conditions, since *Pseudomonas* are suitable to cleave meat protein, after consuming glycose available, promoting meat degradation (SUN; HOLLEY, 2012). To delay microbial growth, a minor proportion of CO_2 can be employed in HiOx MAP (DOULGERAKI et al., 2012). A well-successful and widely used blend is 80% O_2/ 20% CO_2 (80/20). According to Buffo and Holley (2005), HiOx MAP (80/20) may double the shelf-life of chilled meat compared to meat conditioned with air. The use of CO_2 can inhibit the growth of food pathogens and Gram-negative aerobic bacteria, since they are more sensitive to CO_2 than Gram-positives (DANIELS et al., 1985). A higher proportion of CO_2 can be used to package meat and prevent aerobic deterioration, with an increment in microbial control (SUN; HOLLEY, 2012).

Despites HiOx MAP increases the acceptance by consumers in relation to meat color (O'SULLIVAN; LE FLOCH; KERRY, 2015), this atmosphere has been associated with some loss in meat overall quality, by promoting off-odor and off-flavor, attributable to lipid oxidation, especially polyunsaturated phospholips present in cellular membrane, for example (JAYASINGH et al., 2002). In terms of sensory quality, Jayasingh et al. (2002) reported consumers deprecated HiOx displayed

ground beef, with respect to flavor, texture and juiciness and overall acceptability. Seyfert et al. (2006) also found increased TBARS value after packaging and displaying of beef in HiOx MAP.

Besides organoleptic defects, lipid oxidation has been associated with beef discoloration, by oxidation of myoglobin (formation of MMb) (CHAN et al., 1997). Lipid oxidation can initiate the conversion of OMb to MMb. According to Ramanathan et al. (2012) and Suman et al. (2014), beef myoglobin is greatly susceptible to nucleophilic attack by ROS and by aldehydes generated from peroxidation, such as 4-hydroxynonenal, which increases the proportion of MMb (Fe^{3+}) on beef surface.

2.5. Color methods for different pH_u ranges in fresh beef

Because of the difference in pH_u, some research methods to evaluate meat color in high pH_u beef samples require adaptations or a new approach in order to achieving reliable data. Here we discussed on some methods which are employed in color stability research and compare them with standard methods applicable for normal pH_u, present in AMSA Meat Color Measurement Guidelines (AMSA, 2012): myoglobin quantification, NO-MRA, and OCR.

There are two protocols to quantify total myoglobin of fresh and cooked meat in AMSA Meat Color Measurement Guidelines (AMSA, 2012): based on the isobestic spectrophotometric point and by reducing all myoglobin forms to DMb. Both strategies can be employed by researchers to high pH_u. However, the extraction of the pigment with neutral buffer (40 mM potassium phosphate, pH 6.8) is hampered in high pH meat, not resulting in total pigment extracted and, thus, in a pink color remaining in the centrifuge pellet after one extraction. In order to improving pigment extraction, an acidic buffer can be chosen, as used by deDUVE (1948) and by Hunt and Hedrick (1977), adapted from Poel's myoglobin quantification method. The use of acidic buffer (0,01 N, Hunt and Hedrick 1977, or 800 mM, McKeith et al. 2016, sodium acetate, pH 4.5) compensates the native higher pH of the muscle, also observed in pre-rigor muscle (abdominal muscle from biopsy) observed by deDuve (1948). The repetition of extraction step ensures the removal of myoglobin from tissue, as followed by Hunt and Hedrick (1977) and McKeith et al. (2016).

NO-MRA method is based on the complete oxidation of myoglobin in nitric oxide solution (0.3% for 20 min at 25 ºC) with subsequent myoglobin reduction under vacuum condition at 20-30 ºC during 2h. The estimation of the proportion of each myoglobin redox form is achieved by scanning samples with a Hunter Miniscan colorimeter with settings previously described that had been calibrated through the O_2 impermeable film of a vacuum bag. The complete oxidation may be estimated by the ratio of specific wavelengths (572nm/525nm), being the proportion of each myoglobin redox form confirmed by the ratio 630nm/580nm. Another protocol to estimate MMb redox form on the samples surface is based on the creation of reference standards for 100% MMb and DMb. Thus, the

proportion of surface MMb is obtained dividing the difference between MMb for 100% DMb and for sample by the difference between MMb from 100% DMb and 100% MMb. However, myoglobin does not oxidize completely in pH_u meat > 5.8, not resulting in 100% MMb samples prior to vacuum-reduction. There is no method in the literature to achieve myoglobin total oxidation. Therefore, the initial MMb formation (IMF) is used by some authors, such as McKeith et al. (2016). IMF represents the proportion of surface MMb formed after myoglobin oxidation in nitric oxide solution.

Likewise MRA, OCR requires a high myoglobin oxygenation (blooming) to forming OMb. In OCR protocol, described by Madhavi and Carpenter (1993), samples are oxygenated during 2h at 2 ºC and subsequently incubated vacuum-packaged during 20-30 min at 25-30 ºC in order to evaluate the oxygen consumption by mitochondria. McKeith et al. (2016) observed that performing OCR in high pH_u, DC samples had lower initial oxygenation (before incubation) than normal pH_u samples, not achieving 100% surface OMb – as also observed by Krzywicki (1979). Therefore, similar to MRA in high pH_u samples, the evaluation of the proportion of deoxygenated OMb was not possible. Therefore, the initial level of OMb was used by authors to indicate the OC.

Calculating MMb, OMb, and DMb proportions on beef surface has been researched by many investigators, such as Krzywick (1979). See AMSA Meat Color Measurement Guidelines (AMSA 2012) to completely understand pigment calculation. However, high pH_u is challenging by disabling a full conversion of these redox myoglobin forms, besides this pH_u range changing muscle structure and water holding capacity. English et al. (2016) estimated DC beef pigments by their reflectance values and showed using the K/S ratios at isobestic points were useful. K/S improves pigment proportion quantification by making data more linear and to account for absorptive (K) and scattering (S) color properties; and its formula is $(1-R)^2/(2R)$, where R is the reflectance obtained by a spectrophotometer (AMSA, 2012).

Despites not having worked with high pH_u, Ramanathan et al. (2010) showed lactate-enhancement in beef had changed overall % reflectance. Nonetheless, 525 nm remained the isobestic point for MMb, OMb, and DMb, and 572, 610, and 473 nm also kept isobestic for MMb, OMb, and DMb, respectively. Therefore, these wavelengths are still useful to calculate surface pigment in high pH_u beef by following formulas: K/S 572 ÷ K/S 525 (MMb), K/S 610 ÷ K/S 525 (OMb), and K/S 473 ÷ K/S 525 (DMb) (AMSA, 2012).

3. MATERIAL AND METHODS

3.1. Raw material and processing

Bulls (30-36 months-old) with great share of Nellore genetics and raised in a pasture fattening regime were slaughtered in a conventional slaughterhouse submitted to Brazilian inspection located in Barretos/SP/Brazil. At about 48 h post mortem at 4 ºC Longissimus lumborum (LL) muscles were deboned from 1st to 6th lumbar vertebra. Eighteen muscles were selected to fit into three pHu groups (pHu, measured at 48 h post mortem) according to Lomiwes et al. (2014) and Contreras-Castillo et al. (2016): normal (n = 6; 5.40 < pHu <5.79), intermediate (n = 6; 5.80 < pHu <6.19) and high (n = 6; pHu > 6.20) by using a portable pH-meter equipped with a Mettler-Toledo penetrating electrode with a thermometer (pH1140 model, Mettler-Toledo, Switzerland). The selected carcasses were deboned vacuum packaged (VSA 211, Cryovac, Sealed Air, NJ, USA) and transported in refrigerated temperature (2°C).

At the pilot plant from the Meat Laboratory at University of São Paulo, in Piracicaba – SP, the muscles were stored at 2 ± 1ºC. On the third day *post mortem* (72h) the superficial fat was removed and then, and all the muscles were cut into 2.5 cm steaks for posterior packaging.

3.2. Packaging

Individual steaks were placed in trays (model 13D65, 24 x 16 x 65 cm, white polypropylene with ethylene vinyl alcohol (EVOH) barrier, O2 permeability < 0.5 cm3/m2/24h at 50% relative humidity, Sealed Air Corp., US). Air was removed from each trays and a high oxygen (HiOx) atmosphere (80% O2/ 20% CO2; Air Liquide Ltd., Brazil) was flushed, with subsequent sealing with a high barrier sealing film (4532-G lid, with nominal thickness of 70 µm, O2 permeability < 5 cm3/m2/24 h at 23ºC, 66% of relative humidity and water vapor permeability < 5 g/m2/24 h at 38ºC e 90% relative humidity, Bemis Company - Dixie Toga, Brazil) by using a Multivac machine (model T200, Multivac, Ltd., Germany).

3.3. Storage and display conditions

All the trays were stored in dark at 2 ± 1 ºC for 5 day before display for 9 days at 2o C under continuous fluorescent light (1,980 ± 150 lux, MLM-1010, Minipa, São Paulo, Brazil)The cold room was defrosted at 4-h intervals. These various storage and display times represent several hypothetical scenarios after processing and before purchasing.

3.4. Analyses

pH, gas composition, instrumental color, and surface pigment were evaluated on days 0 (without MAP), 5 (dark condition), 8 (beginning of light condition), 11 and 14 of steak storage. MRA, OCR and TBARS were determined on days 0, 5 and 14 of display time.

3.4.1. Gas composition

MAP gas composition of each package was measured by using a portable headspace oxygen/carbon dioxide analyzer (CheckPoint®, PBI Dansensor A/S, Denmark) during the display time. This analysis was performed immediately before opening the trays.

3.4.2. pH

pH was determined with a calibrated (pH 4.0, 7.0, and 10.0) portable pH-meter equipped with a Mettler-Toledo penetrating electrode with a thermometer (pH1140 model, Mettler-Toledo, Switzerland). Steaks were measured at three random locations and values were averaged for statistical analyses.

3.4.3. Instrumental color and surface pigments

In the surface of each steak, CIE lightness (L*), redness (a*), yellowness (b*) were read with HunterLab MiniScan XE Plus spectrophotometer (Hunter Associates Laboratory, USA), integrated to an Easy Match QC system, with 2.54 cm diameter aperture, 10º standard observer and illuminant A. Readings were performed immediately after removal of steaks from MAP and at five random locations on steak surfaces of each steak. The spectrophotometer was calibrated using black and white reference standards provided by the manufacturer. With a* and b* values from readings, ratio of a*/b*, hue (arctan (b*/a*)) and chroma $(a^{*2}+b^{*2})^{1/2}$ were calculated.

The proportions of pigments DMb, OMb and MMb were estimated by using selected wavelengths and transforming them into K/S to make data more linear and to account for absorptive (K) and scattering (S) color properties. K/S was obtained by the formula: $(1-R)^2/(2R)$, where R is the reflectance obtained by a spectrophotometer. The selected wavelengths to obtain the pigments were: MMb (KS 572/525), OMb (KS 610/525) and DMb (KS 473/525) (AMSA, 2012). As Hunter spectrophotometer only record reflectance values between 400 and 700 nm at 10 nm intervals, it was necessary to calculate intermediate wavelength values, such as 473 and 572 nm, by linear interpolation.

3.4.4. Initial Metmyoglobin Formed (IMF) and Bloomed Oxymyoglobin

Nitric oxide metmyoglobin reduction was performed according to method present in AMSA (2012). Samples were removed from their trays and a piece 3 x 3 x 2 cm was cut into two halves. The half previously exposed to HiOx MAP was submerged in a 0.3 % (w/v) of sodium nitrite (Sigma Aldrich, MO, USA) for 20 min. Immediately after, the sample was removed from solution, blotted dry and vacuum packaged (Sealed Air, NJ, USA) and scanned on the surface with a HunterLab MiniScan XE Plus spectrophotometer, obtaining 400-700 nm reflectance data. MRA was evaluated by the initial MMb formed in nitric oxide solution, as performed by McKeith et al. (2016).

The inferior half of steak used in MRA analysis – never exposed to oxygen – was used to determine the OCR according to AMSA (2012). The sample was exposed to air for 2 h at 2 ±1 ºC to bloom myoglobin and oxygen-permeable film was used to prevent surface dehydration. After the exposure time, sample was vacuum-packaged (Sealed Air, NJ, USA) and scanned with a HunterLab MiniScan XE Plus spectrophotometer, obtaining 400-700 nm reflectance data. Bloomed OMb was used to estimate OCR as performed by McKeith et al. (2016).

3.4.5. Lipid oxidation

On days 0, 5 and 14 of retail display, lipid oxidation was analyzed in triplicate measuring the thiobarbituric acid reactive substances (TBARS), according to the method of Vynche (1970, 1975) and Sørensen and Jørgensen (1996). Five grams of ground beef (processor Walita Master, 600 W) was placed in a 50 mL polypropylene tube and homogenized with 15 mL tricholoroacetic acid (7,5%)/propyl gallate (0,1%)/EDTA (0.1%) solution using a Ultra Turrax (40s, 3200rpm, IKT, T18 Basic Willmington, North Carolina) for 40 s at 3200 rpm. The meat homogenate was filtered in filter in qualitative filter paper (12.5 mm, Whatman). Five mL of filtrate were placed in test tube with screw cap, along with 5 mL 2-thiobarbituric acid (0.02 M). The mixture was vortexed for 10 s and then incubated in a 95 ºC water bath for 40 min. After cooling at room temperature, the samples were vortexed before absorbance reading at 532 nm and 600 nm, using a UV-Vis spectrophotometer (Shimadzu UV mini 1240, Shimadzu Corporation Japan). A blank composed by ultrapure water was read. TBARS was calculated from a 1,1,3,3-tetraethoxypropane standard curve.

3.4.6. Statistical analysis

The study was conducted on a factorial scheme (3 × 5), considering 3 pH_u ranges in 5 storage times (0, 5, 8, 11 and 14 days). Experimental unit in the study was the sampled muscle from animal, composed by six repetitions of each pH_u range.

Variables were analyzed using the Univariate Procedure of SAS (SAS, Inst. Inc., Cary, NC, US). At this stage the proc Univariate was used for statistical analysis and analyzed using MIXED Procedure of SAS, considering time (storage days) as repeated measure on the model. All the effects from the model were assumed as fixed (pH_u ranges, time and its interactions). After ANOVA, least square means were estimated as well as their standard errors (SE). Tukey test was used to compare the average differences between pH_u ranges, time and its interactions.

Principal Component Analysis (PCA) was carried out to identify relationships between ultimate pH ranges and color stability parameter. PCA was performed using XLSTAT, an Excel add-in by Microsoft®. PCA was type Pearson the biplot based on the Euclidian distance among variables.

4. RESULTS AND DISCUSSION

4.1. pH measurement

At 48h postmortem, muscle pH ranged between 5.40 and 6.76. pH measurements performed at each steak during display-time is shown in Fig. 2. Storage did not affect pH across days for any treatment. Nevertheless, the difference among all three groups occurred on day 0 only ($p < 0.0001$). From day 5, intermediate and normal groups did not present statistical difference but had lower values than high pH_u ($p < 0.0001$), as expected.

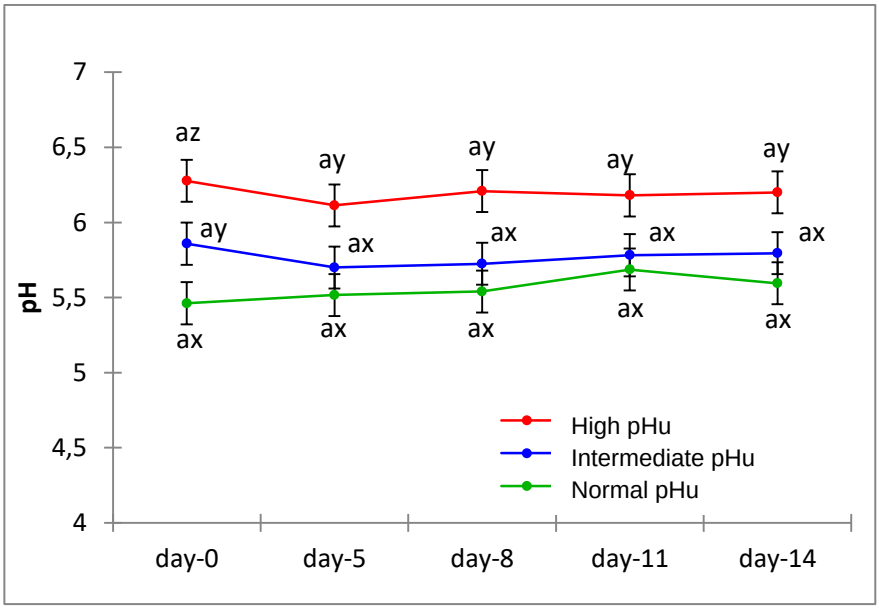

Fig. 2. pH evolution during display-time of normal, intermediate and high pH_u groups in HiOx MAP (80/20). [ab] Means with different letters across days ($P < 0.05$). [xy] Means with different letters across pH_u ranges ($P < 0.05$)

4.2. Gas composition

Gas composition was altered during time and across pHu group. Fig. 3 shows the accumulation of oxygen (O_2, Fig. 3A) and carbon dioxide (CO_2, Fig 3B) for the three treatments. High pHu differed from other two other treatments and presented less O_2 and more CO_2 in trays headspace ($p < 0.05$). During display-time, O_2 concentration in high pHu trays decreased faster than for others pHu ranges; from 80.82% d 0 to 77.52% d 14 ($p < 0.05$). CO_2 concentration increased more in high pHu trays than in the other ranges; from 21.48% d0 to 24.95% d14 ($p < 0.0001$). These changings may be related to cellular respiration by muscle and microorganism cells. Bendall and Taylor (1972) reported mitochondrial oxygen consumption is pH-dependent. As pH increases, the oxygen demand intensify, which promotes diminution of O_2 and increase of CO_2 over the retail time in HiOx trays.

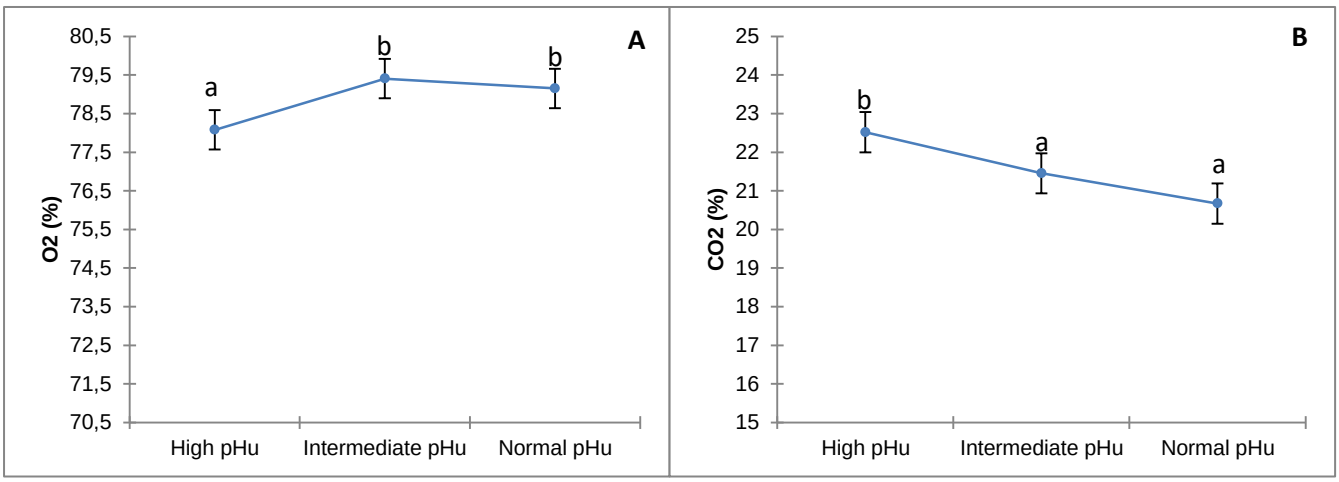

Fig. 3. Oxygen (O_2, 3A) and carbon dioxide (CO_2, 3B) proportion in trays for normal, intermediate and high pH_u ranges of beef packed in high-oxygen modified atmosphere (HiOx MAP 80/20). [ab] Means with different letters across pH_u ranges ($P < 0.05$)

4.3. Instrumental color

Retail time and pHu ranges affected instrumental color attributes CIE a*, b*, a*/b*, Hue, and Chroma, with significant interactions p < 0.05, according to Table 2. However, lightness (L*) did not change throughout the display time and did not present significant interaction.

Table 2. Sources of variation for instrumental color parameters.

	Effects		
	T	D	TxD
L*	53.33***	1.17 n.s.	1.65 n.s.
a*	3.27*	10.21***	8.65***
b*	16.74***	17.95***	8.09***
a*/b*	68.99***	18.78***	5.61***
Hue	76.07***	16.98***	4.26**
Chroma	4.10*	13.06***	8.71***

n.s.: not significant; *: $P<0.05$; **: $P < 0.01$; ***, $P < 0.0001$; L*: lightness; a*: redness; b*: yellowness; T: ultimate pH ranges (normal: 5.40 – 5.79; intermediate: 5.80 – 6.19; high: > 6.20); D: display time (0, 5, 8, 11, and 14 days in high-oxygen modified atmosphere package).

Time evolution of CIELAB color and pigment parameters after storage and display time in HiOx trays are reported in Table 3.The results show that high pHu group were darker than normal and intermediate pHu ranges on each display time ($p < 0.05$). The large effect of changes of pH on beef color is well known (ABRIL et al., 2001; ENGLISH et al., 2016a; HUNT; HEDRICK, 1977; PAGE; WULF; SCHWOTZER, 2001). Lower L* ($p < 0,05$) in high pHu group is in agreement with reported by Abril and others (2001), McKeith and others (2016), and English and others (2016), and it has been associated with a higher water-holding capacity of miofibrilar proteins (HUGHES et al., 2014) and with larger muscle fiber width, which results in short space between fibers and, consequently (HUGHES et al., 2017). Both factors result in decreased light scattering inside the structure of fiber, as described by MACDOUGALL (1982). Intermediate pHu was statistically similar to normal pHu group, decreasing L* values only after day 11.

High pH_u steaks had an initial lower a* ($p < 0.05$), may be attributed to a higher mitochondrial respiration in high pHu, which decreases surface OMb by competing for the content of oxygen (BENDALL; TAYLOR, 1972; TANG et al., 2005), as observed at surface OMb result in this study. Redness (a*) presented a slight decrease for normal pHu after exposition to fluorescent light (day 5) (Table 3). Intermediate and high pH_u steaks had an increasing in a* after HiOx packaging ($p < 0.05$). Despite the formation of the thick layer of OMb is impaired due to the intense mitochondrial activity, the high partial pressure of oxygen inside the tray may have favored beef oxygenation, increasing a* after packaging. There was a falling on a* during time in normal pHu, as reported by Knock et al. (2006) for control group.

Treatment groups differed only on day 0 for yellowness (b*) ($p < 0.05$), as observed in Table 3. Values of b* showed an increasing between days 0 and 5 ($p < 0.05$), stabilizing afterwards, for intermediate or high pHu ranges. For normal pHu, on the other hand, b* was stable during all the time. Knock et al. (2006) also did not observe changing in b* values for normal pHu beef packaged in HiOx MAP during 14 days. The least b* value in high pHu on day 0 may be attributed to a greater oxygen consumption rate (OCR) this pHu range has, according to Bendall and Taylor (1972). So, this little yellowness could result in a purplish color due to surface accumulation of DMb.

To evaluate discoloration during display life, the ratio a*/b*, hue angle and Chroma should be considered, as shown in Table 3 (AMSA, 2012). Ratio a*/b* and C* has an inversely relation to discoloration and larger values of the hue angle indicate less red colors. Higher ratio a*/b* values ($p < 0.05$) were observed for high pHu on days 0, 8, 11, and 14 than for normal and intermediate pHu ranges. Ratio a*/b* gradually decreased along time for all pH groups. Hue data are in agreement with the ratio results and showed as the least among treatments ($p < 0.05$), as observed Abril and others (2001) and Stackhouse and others (2016). The normal group had more increase along time than intermediate and high pHu ($p <0.05$). More stability can be related to the less oxidative susceptibility presented by myoglobin in higher pH values (HUNT; SØRHEIM; SLINDE, 1999).

After 5 days in HiOx packaging, chroma increased significantly ($P < 0.05$) in high pH group indicate improvement of color by HiOx packaging in beef which has less oxygenation at natural

conditions (in the air) and was stable until day 14 when having no difference among groups (exception on day 11, when intermediate group had more Chroma, $p < 0.05$).

Table 3. Least square means for instrumental color parameters from beef longissimus lumborum with 3 pHu ranges during beef steaks storage in HiOx MAP.

Attribute	pH_u	Display-time (day)					SE
		0	5	8	11	14	
L*	Normal	49.20^{y}	47.93^{y}	47.68^{y}	50.64^{y}	47.77^{y}	1.79
	Intermediate	47.99^{y}	48.86^{y}	47.75^{y}	47.19^{xy}	47.00^{xy}	
	High	41.49^{x}	43.21^{x}	42.65^{x}	44.50^{x}	44.10^{x}	
a*	Normal	25.84^{by}	26.16^{b}	22.37^{abx}	21.91^{ax}	23.77^{abx}	1.85
	Intermediate	23.29^{ay}	26.87^{b}	25.07^{bxy}	26.08^{by}	26.20^{bxy}	
	High	17.34^{ax}	27.24^{b}	26.23^{by}	26.37^{by}	27.13^{by}	
b*	Normal	17.64^{y}	17.96	16.72	16.54	17.92	1.37
	Intermediate	15.63^{ay}	18.96^{b}	18.35^{b}	18.51^{b}	18.70^{b}	
	High	9.09^{ax}	18.05^{b}	16.12^{b}	17.37^{b}	17.37^{b}	
Redness (a*/b*)	Normal	1.47^{bx}	1.46^{b}	1.34^{ax}	1.32^{ax}	1.32^{ax}	0.05
	Intermediate	1.49^{bx}	1.42^{b}	1.37^{ax}	1.40^{ax}	1.40^{ax}	
	High	1.90^{by}	1.51^{a}	1.63^{aby}	1.52^{ay}	1.56^{ay}	
Hue	Normal	33.28^{ay}	34.49^{ab}	36.74^{bcy}	37.15^{cy}	37.06^{cy}	1.15
	Intermediate	33.83^{ay}	35.50^{ab}	36.20^{by}	35.41^{abxy}	35.56^{aby}	
	High	27.70^{ax}	33.49^{b}	31.71^{bx}	33.29^{bx}	32.60^{bx}	
Chroma	Normal	31.29^{y}	31.73	27.96	27.46^{x}	29.77	2.22
	Intermediate	28.06^{ay}	32.88^{b}	31.08^{ab}	31.99^{aby}	32.20^{ab}	
	High	19.59^{ax}	32.68^{b}	30.82^{b}	31.59^{bxy}	32.21^{b}	

L*: lightness; a*: redness; b*: yellowness; T: ultimate pH ranges (normal: 5.40 – 5.79; intermediate: 5.80 – 6.19; high: > 6.20); ^{abc} Least square means with different letters in the same row are different ($P < 0.05$) for each attribute. ^{xyz} Least square means with different letters in the same column are different ($P < 0.05$) for each attribute, SE: Standard Error

4.4. Surface pigments

DMb, MMb and OMb were estimated by the ratio of reflectance KS/ 473/ 525 nm, KS 572/ 525 nm, and KS 610/ 525 nm, respectively and its evolution throughout display time is showed in Fig. 4. The interpretation of these results indicates the higher is the value of the ratio; the lower is the accumulation of the pigment on the surface of steaks. The pHu ranges affected the onset of surface MMb and OMb ($p < 0.05$), whereas storage affected all three pigments ($p < 0.05$), with interaction between time and range for all redox forms ($p < 0.05$).

At the beginning of display (Fig. 4), high pHu group showed more DMb than normal and intermediate ranges. After packaging, DMb proportion decreased ($p <0.05$) and no difference was observed between day 5 and 14. Other both treatments did not have difference throughout time. Due to DMb reduction, all three pigments were statistically similar on days 5, 8 and 11. On day 14, normal group had more surface DMb than high treatment ($p < 0.05$).

Initially, steaks had a lower proportion of MMb, which is expected due to the lower myoglobin oxidation, as observed in Fig. 4. During cold storage under fluorescent light, to simulate the retail condition, MMb increased in normal and intermediate groups. Normal treatment showed an intense increase, especially after light period (1.29 d 0^{a} - 1.20 d. 8^{bc} – 1.17 d 14^{c}) ($p <0.05$), whereas intermediate range had a moderate increment in MMb proportion during the whole period (1.30 d 0^{a} – 1.20 d 8^{b} – 1.21 d 14^{b}) ($p< 0.05$). High pHu did not have difference throughout time, showing no significant myoglobin oxidation in a high-oxygen medium. In relation to the treatments, a difference was observed after day 8. On day 8, normal and intermediate groups had more MMb than high pHu range ($p < 0.05$), whereas on day 11, intermediate was similar to high pHu and both were lower than normal group ($p <0.05$). On day 14, there was no difference across treatments, as expected due to a numerical increase in MMb proportion in high pHu.

Due to a greater OCR observed in high pH by Bendall and Taylor (1972), the surface proportion of OMb decrease as pH increases. This phenomenon was observed on day 0 (Fig. 4), when high pHu had less OMb than other both groups ($p < 0.05$). After packaging in HiOx, all treatments did not show difference during display time with exception of day 11, when normal group had less OMb than other both treatments. Throughout storage, normal presented a decrease in OMb (0.20 d 0 – 0.26 d 11), whereas OMb proportion in intermediate and high was stable after day 5, when there was an increasing. Fig. 5 shows a transversal section of a High pHu steak packed in HiOx and stored for 8 days.

Dunne, Monahan and Moloney (2011) stated in their review on physical activity affecting beef darkening that pasture-finishing leads to a higher pH and darker beef, which were observed in the present study and would not oxygenate properly. High-oxygen atmosphere, on the other hand, resulted in high proportion of OMb and low of DMb on the surface of high pHu steaks during 14 days under refrigeration.

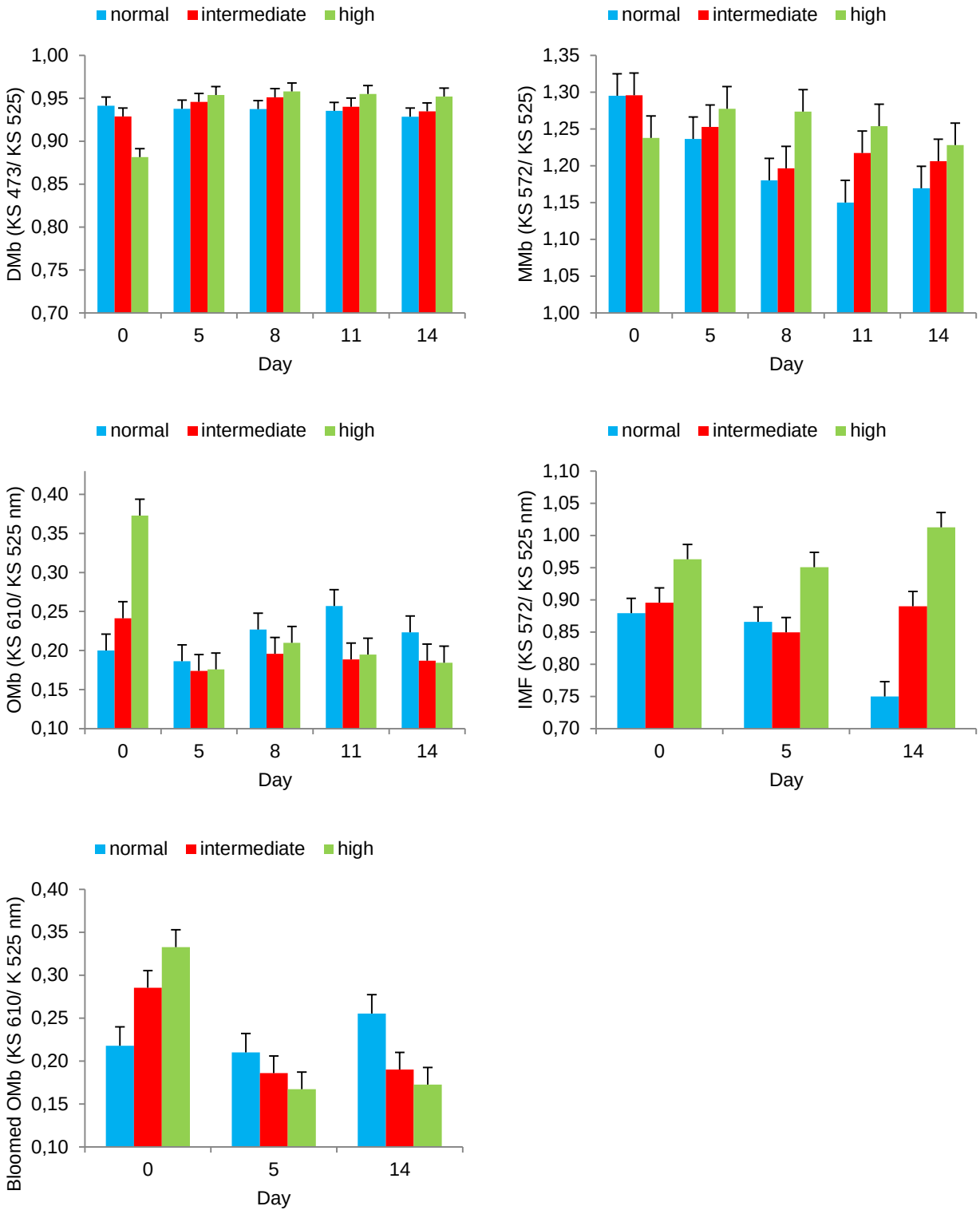

Fig. 4. Effect of beef ultimate pH on surface pigments (DMb, MMb, and OMb), MRA (IMF) and OCR (bloomed OMb) throughout 14 d-retail time in high-oxygen modified package 80% O_2/ 20% CO_2.

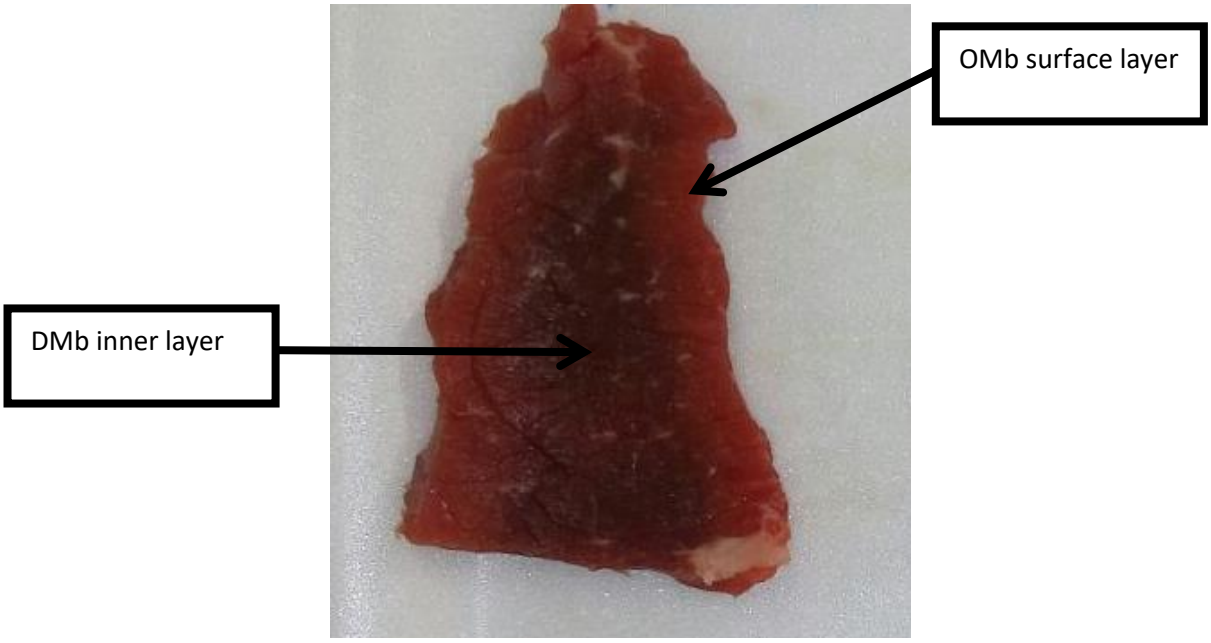

Fig. 5. Transversal section of a high pHu steak stored in HiOx for 8 days at 2 ºC.

4.5. Initial Metmyoglobin Formed (IMF) and Bloomed Oxymyoglobin

Initial metmyoglobin formed (IMF) is an indicator of muscle MMb reducing ability (MRA), as reported by McKeith et al. (2016). According to Fig. 4, less MMb was formed in high pHu throughout display time ($p < 0.05$), which indicates these samples had more MRA than other 2 groups. Intermediate pHu range had less MMb than normal group on day 14 ($p < 0.05$). Normal group had a decrease in MRA over the time, showing more MMb on day 14 than on days 0 and 5 ($p < 0.05$), whereas intermediate group was stable during time and high pHu presented less MMb on day 14, which corroborates higher pH_u are less susceptible to myoglobin oxidation (GEORGE; STRATMANN, 1952; MCKEITH et al., 2016). MMb reductase is the main component of the enzymatic MRA and its activity increases in function to medium pH, achieving its maximum activity at pH 7.3 (ECHEVARNE; RENERRE; LABAS, 1990). Ramanathan et al. (2012) reported that at pH 7.4 a mitochondrial NADH-dependent reductase reduced significantly more MMb in control group than at pH 5.6.

The least MMb formed in MRA analysis in high pHu group is in concordance with other findings in this study, such as higher redness (more a* and a*/b*) and lower discoloration throughout storage (less Hue and surface MMb), It demonstrates the oxidative protection of high pHu regarding red color stability, as authors have been documented (ABRIL et al., 2001; CHMIEL et al., 2012; ENGLISH et al., 2016; GEORGE; STRATMANN, 1952; MCKEITH et al., 2016; STACKHOUSE et al., 2016; WULF; WISE, 1999).

McKeith et al. (2016) found dark-cutters (pH > 6.1) had greater content of mitochondria and discussed these mitochondria could provide reducing equivalents for mitochondrial pyridine nucleotide reduction, increasing NADH concentration in cell. Unpublished data of our group showed High pHu

group had more NADH concentration than normal group. It is known NADH acts as coenzyme for the NADH-cytochrome b5 MMb reductase and higher NADH content increases and extends color stability by preventing myoglobin oxidation. Kim et al. (2009b) showed NADH is formed by oxidation of lactate by lactate-dehydrogenase and, this coenzyme acted as an intermediate at MMb reduction to DMb. The greater content of NADH in the high pHu group could also be a factor for the increased MRA this group had in this study.

Oxygen consumption rate was determined by bloomed OMb and its evolution throughout time is showed in Fig. 4. On day 0, the greater the pHu, the less OMb was formed ($p < 0.05$). This was expected since as pH increases, there is a more pronounced mitochondrial activity, which oxidizes molecular oxygen in electron-transport chain (BENDALL; TAYLOR, 1972; TANG et al., 2005). As a result, the deoxygenation of red OMb to a purplish DMb is favoured in high pHu, as showed in surface OMb and DMb (Fig. 4) and in b*(Table 3) in high pHu on day 0, and observed by McKeith et al. (2016).

After packaging in HiOx MAP (day 5), bloomed OMb proportion increased, indicating OCR decreased significantly in intermediate and high groups ($p < 0.05$), remaining its proportion on day 14. As a result of this reduction in these both treatments, normal group exhibited more OCR in the end of display period ($p < 0.05$). This reduction in OCR may be attributed to a normal decline at the active mitochondrial respiration post-mortem. However, Ashmore, Parker, and Doerr (1972) found dark-cutting beef presented higher OCR, which extended for longer time than normal pHu beef. In this study, OCR in the normal pHu samples was stable during 14 days in HiOx MAP.

Oxygen consumption has been inversely associated with color stability by decreasing of oxygen level and formation of reactive oxygen species (ROS) in mitochondrial respiration (RENERRE, 1990; RENERRE; LABAS, 1987). OCR data for the high treatment in HiOx MAP shows lower L* for these samples are more linked to a low light scattering than to a high oxygen consumption.

4.6. Lipid oxidation

Statistical analysis showed significant differences for pH_u ranges, display-time, and interaction between pH_u range and display-time ($p < 0.05$). An increase of oxidation throughout the display time can be observed in Fig. 6 and it was also indicated by Knock et al. (2006). There was no statistical difference between days 0 (before packaging) and 5 (before displaying under fluorescent light) for all pH_u ranges. After light-exposing, on the other hand, there was a significant increase in TBARS values, which is related to photo-oxidation.

High pH_u exhibited great lipid stability, since this range does not change throughout the period. TBARS in normal and intermediate groups increased on day 14, after 9 day under fluorescent-light and in HiOx atmosphere. The higher oxidative stability of high pH_u has been reported by many

authors (KESKINEL; AYRES; SNYDER, 1964; SHAREDEH et al., 2015) and is associated to the least oxidation of myoglobin to forming MMb. As pH decreases to 5.6, more oxidation is observed. A mechanism proposed is based on a more pronounced oxygen release from heme iron as hydroperoxide and superoxide radicals and iron release from Fe-carrying proteins, such as ferritin and transferrin) as pH decreases (BERGAMASCHI; PIZZA, 2011; SHAREDEH et al., 2015). The free iron acts as catalyzer to production of superoxide and hydroxide radicals, both greatly pro-oxidants to lipids and proteins (SUGAWARA; SHIKAMA, 1980).

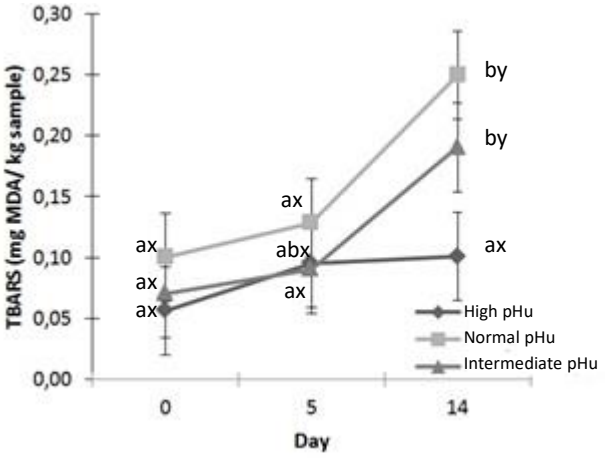

Fig. 6. Increase of lipid oxidation for low (normal), intermediate and high pH_u groups during time of displaying in packages HiOx MAP (80/20). ^{ab} Means with different letters across days ($P < 0.05$). ^{xy} Means with different letters across pH_u ranges ($P < 0.05$)

4.7. Principal Component Analysis (PCA)

According to PCA in Fig. 7, high pHu differed from normal and intermediate groups in relation to the first factor in the distance biplot (67.63% of the variance). However intermediate and normal treatments were not close, being distinguished by the second factor (32.27% of the variance). This behavior is frequently observed in this study. Therefore, PCA showed there were three distinct pHu ranges in the present study.

Color studies have showed pH_u grouping into ranges is not consensus, which has resulted in several classifications, according to each research group. Abril et al. (2001) determined the effect of pHu on meat color parameters and performed a cluster analysis and observed variables acted as 2 groups ($pH < 6.1$ and $pH \geq 6.1$).

The biplot present in Fig. 7 shows variables distributed through the three pHu ranges. Normal group had relation greatly to lipid oxidation (TBARS) and moderately to OCR. Increased lipid oxidation was observed in normal group due to lower oxidative stability this range possesses - also present in myoglobin oxidation data. OCR may have been related to normal group due to the intense decrease on days 5 and 14 in intermediate and high pHu ranges.

Intermediate pHu range was associated with headpace O2, L*, b*, Hue, and Chroma. Although several authors have indicated pH > 5.8 has improved color stability (PAGE; WULF; SCHWOTZER, 2001; VILJOEN; DE KOCK; WEBB, 2002), intermediate group in this study (pHu = 5.80 – 5.19) did not present results similar to high pHu range. Most of the analyses, normal and intermediate group had no statistical difference. In the end of display time, intermediate presented similarity to both other treatments, such as for L* (d 11 and 14) and hue (d 11), besides a* (d 8 and 14), MMb (d 11), and OMb (d 11) which were not close in PCA.

High group had great relationship with headpace CO2, a*/b*, IMF, pH, and KS 572/525, as expected. As observed in this study and demonstrated by authors (ENGLISH et al., 2016; MCKEITH et al., 2016), high pHu range showed more pronounced MRA (measured as IMF), resulting in the least surface MMb (KS 572/525) and the highest redness (a*/b*) throughout display time. Despite not being so close, K/S 610/525 was associated with high pHu, indicating this group may have had lower proportion of OMb. Since high group had similar surface OMb to other both groups, this associated may be attributed to day 0 of display life, when high showed the least surface OMb.

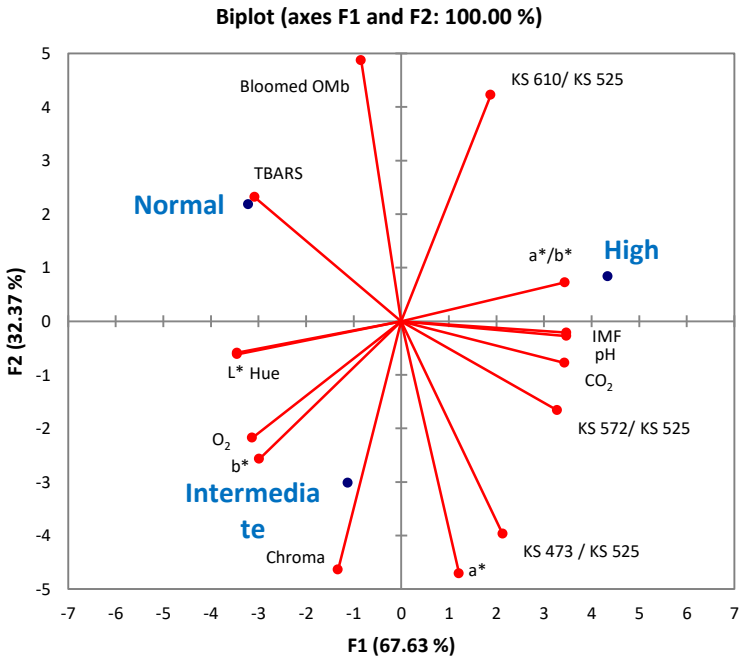

Fig. 7. Distance biplot of principal component analysis (PCA) relating pHu groups and color and lipid stability variables.

5. CONCLUSION

High pHu beef (> 6.20) from Nellore bulls were darker, redder and had improved surface color during storage in atmosphere 80/20% O2/CO2, in comparison with normal pH_u (5.40 – 5.79). Intermediate group, pHu between 5.80 and 6.19, seemed to have as advantages being lighter higher range, according to L* results, and having a long color stability, according to MMb and IMF results. Hence, ultimate pH affected color stability in pasture-fed Nellore beef.

Packaging steaks in HiOx MAP improved color parameters in high pHu group, comparing between days 0 and 5 of display-time. Therefore, despite beef with pHu > 5.8 has been related to darker cutters, HiOx showed as a strategy to improved red color.

High pHu showed greater lipid oxidation stability even in a medium with a high content of oxygen and under fluorescent light for 14 days at 2 ºC.

REFERENCES

ABRIL, M. et al. Beef colour evolution as a function of ultimate pH. **Meat Science**, Barking, v. 58, n. 1, p. 69–78, 2001.

AMSA – AMERICAN MEAT SCIENCE ASSOCIATION. **Meat color measurement guidelines. Champaign**, Illinois, 2012. 125p.

ARIHARA, K. et al. Localization of metmyoglobin-reducing enzyme (NADH-cytochrome b5 reductase) system components in bovine skeletal muscle. **Meat Science**, Barking, v. 39, n. 2, p. 205–213, 1995.

ASHMORE, C. R. et al. Respiration of mitochondria isolated from dark-cutting beef. **Journal of Animal Science**, Champaign, v. 33, n. 3, p. 574–577, 1971.

ASHMORE, C. R.; PARKER, W.; DOERR, L. Respiration of mitochondria isolated from dark-cutting beef: postmortem changes. **Journal of Animal Science**, Champaign, v. 34, n. 1, p. 46-48, 1972.

BCRC - BEEF CATTLE RESEARCH COUNCIL. National Beef Quality Audit. Retrieved April 20, 2017, from http://www.beefresearch.ca/research-topic.cfm/beef-quality-audits-40#darkcutting.

BEKHIT, A. E. D.; FAUSTMAN, C. Metmyoglobin reducing activity. **Meat Science**, Barking, v. 71, n. 3, p. 407–439, 2005.

BELSKIE, K. M. et al. Reverse electron transport effects on NADH formation and metmyoglobin reduction. **Meat Science**, Barking, v. 105, p. 89–92, 2015.

BENDALL, J. R.; TAYLOR, A. A. Consumption of oxygen by the muscles of beef animals and related species. II. Consumption of oxygen by post-rigor muscle. **Journal of the Science of Food and Agriculture**, London, v. 23, n. 6, p. 707–719, 1972.

BERGAMASCHI, M.; PIZZA, A. Effect of pork meat pH on iron release from heme molecule during cooking. **Journal of Life Sciences**, New Delhi, v. 5, p. 376–380, 2011.

BRAY, A. R.; GRAAFHUIS, A. E.; CHRYSTALL, B. B. The cumulative effect of nutritional, shearing and pre-slaughter washing stresses on the quality of lamb meat. **Meat Science**, Barking, v. 25, n. 1, p. 59–67, 1989.

BRODY, A. L. Case-ready packaging for red meat. **Food Technology**, Chicago, v. 61, p. 70-71, 2007.

BUFFO, R. A., HOLLEY, R. A. Centralized packaging systems for meats. In: Han J.H, editor. **Innovations in food packaging**. 1st Ed. Oxford: Elsevier, 2005, p. 227–36.

CANTO, A. C. V. C. S. et al. Color attributes and oxidative stability of longissimus lumborum and psoas major muscles from Nellore bulls. **Meat Science**, Barking, v. 121, p. 19–26, 2016.

CHAN, W. K. M. et al. Lipid oxidation induced by oxymyoglobin and metmyoglobin with involvement of H_2O_2 and superoxide anion. **Meat Science**, Barking, v.46, p.181-190, 1997.

CHMIEL, M. et al. Application of a computer vision system to classify beef as normal or dark, firm, and dry. **Journal of Animal Science**, Champaign, v. 90, n. 11, p. 4126–4130, 2012.

CHOI, Y. M.; KIM, B. C. Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. **Livestock Science**, Amsterdam, v. 122, n. 2–3, p. 105–118, 2009.

CONTRERAS-CASTILLO, C. J. et al. The effect of electrical stimulation on post mortem myofibrillar protein degradation and small heat shock protein kinetics in bull beef. **Meat Science**, Barking, v. 113, p. 65–72, 2016.

DANIELS, J. A. et al. A Review of Effects of Carbon Dioxide on Microbial Growth and Food Quality. **Journal of Food Protection**, Des Moines, v. 48, n. 6, p. 532-537, 1985.

DE DUVE, C. A spectrophotometric method for the simultaneous determination of myoglobin and hemoglobin in extracts of human muscle. **Acta Chemica Scandinavica**, Copenhagen, v. 2, p. 264-289, 1948.

DEVOL, D. L. et al. Comparison of M. longissimus dorsi pigment concentration from implanted and control Angus and Limousin bulls and steers. **Meat Science**, Barking, v. 14, n. 3, p. 165–173, 1985.

DJENANE, D, SÁNCHEZ-ESCALANTE, A, BELTRÁN, J. A., RONCALÉS, P. Extension of the shelf-life of beef steaks packaged in a modified atmosphere by treatment with rosemary and displayed under UV-free lighting. **Meat Science**, Barking, *v.* 64, p. 417–26, 2003.

DOULGERAKI, A. I. et al. Spoilage microbiota associated to the storage of raw meat in different conditions. **International Journal of Food Microbiology**, Amsterdam, v. 157, p. 130-141, 2012.

DUFRASNE, I. et al. Effects of a grazing period on performance of finishing bulls: comparison indoor finishing system**. Animal Science**, New York, v. 60, n. 1, p. 75-80, 1995.

DUNNE, P. G. et al. Colour of muscle from 18-month-old steers given long-term daily exercise. **Meat Science**, Barking, v. 71, n. 2, p. 219–229, 2005.

DUNNE, P. G.; MONAHAN, F. J.; MOLONEY, A. P. Current perspectives on the darker beef often reported from extensively-managed cattle: Does physical activity play a significant role? **Livestock Science**, Amsterdam, v. 142, n. 1–3, p. 1–22, 2011.

ECHEVARNE, C.; RENERRE, M.; LABAS, R. Metmyoglobin reductase activity in bovine muscles. **Meat Science**, Barking, v. 27, n. 2, p. 161–172, 1990.

ENGLAND, E. M. et al. Excess glycogen does not resolve high ultimate pH of oxidative muscle. **Meat Science**, Barking, v. 114, p. 95-102, 2016.

ENGLAND, E. M. et al. pH inactivation of phosphofructokinase arrests postmortem glycolysis. **Meat Science**, Barking, v. 98, n. 4, p. 850-857, 2014.

ENGLISH, A. R. et al. Effects of aging on the fundamental color chemistry of dark-cutting beef. **Journal of Animal Science,** Champaign, v. 94, p. 4040–4048, 2016.

FERGUSON, D. M.; WARNER, R. D. Have we underestimated the impact of pre-slaughter stress on meat quality in ruminants? **Meat Science**, Barking, v. 80, n. 1, p. 12–19, 2008.

FERRAZ, J. B. S.; FELÍCIO, P. E. DE. Production systems - An example from Brazil. **Meat Science**, Barking, v. 84, n. 2, p. 238–243, 2010.

GEORGE, P.; STRATMANN, C. J. The oxidation of myoglobin to metmyoglobin by oxygen. I. **The Biochemical journal**, London v. 51, n. 1935, p. 103–108, 1952.

GRABER, S. G.; WOODWORTH, R. C. Myoglobin expression in L6 muscle cells. Role of differentiation and heme. **Journal of Biological Chemistry**, Baltimore, v. 261, n. 20, p. 9150–9154, 1986.

GRABEZ, V. et al. Protein expression and oxygen consumption rate of early postmortem mitochondria relate to meat tenderness. **Journal of Animal Science**, Champaign, v. 93, n. 4, p. 1967–1979, 2015.

HOLDSTOCK, J. et al. The impact of ultimate pH on muscle characteristics and sensory attributes of the longissimus thoracis within the dark cutting (Canada B4) beef carcass grade. **Meat Science**, Barking, v. 98, n. 4, p. 842–849, 2014.

HUGHES, J. M. et al. A structural approach to understanding the interactions between colour, water-holding capacity and tenderness. **Meat Science**, Barking, v. 98, n. 3, p. 520–532, 2014.

HUGHES, J. M. et al. High pH in beef longissimus thoracis reduces muscle fibre transverse shrinkage and light scattering which contributes to the dark colour. **Food Research International,** Ottawa v. 101, p. 228-238, 2017.

HUGHES, J. M., KEARNEY, G., WARNER, R. D. Improving beef meat colour scores at carcass grading. **Animal Production Science**, Melbourne, v. 54, n. 4, p. 422-429, 2014.

HUNT, M. C.; HEDRICK. Chemical, physical and sensory characteristics of bovine muscle from four quality groups. **Journal of Food Science**, Chicago, v. 42, n. 3, p. 716–720, 1977.

HUNT, M. C.; SØRHEIM, O.; SLINDE, E. Color and Heat Denaturation of Myoglobin Forms in Ground Beef. **Journal of Food Science**, Chicago, v. 64, n. 5, p. 847–851, 1999.

IBGE. **Indicadores IBGE: Estatística da produção pecuária**. 2017. Retrieved May 19, 2017, from: <ftp://ftp.ibge.gov.br/Producao_Pecuaria/Fasciculo_Indicadores_IBGE/abate-leite-couro-ovos_201604caderno.pdf>.

IBGE. **Indicadores IBGE: Produção da Pecuária Municipal**. 2015. Retrieved Jan 20 2017, from < http://www.ibge.gov.br/home/estatistica/economia/ppm/2015/>.

IMMONEN, K. et al. Glycogen concentrations in bovine longissimus dorsi muscle. **Meat Science**, Barking, v. 54, n. 2, p. 163–167, 2000.

JAYASINGH, P. et al. Sensory Evaluation of Ground Beef Stored in High-oxygen Modified Atmosphere Packaging. **Journal of Food Science**, Chicago, v. 67, n. 9, p. 5–8, 2002.

KANDEEPAN, G. et al. Feeding regimens affecting meat quality characteristics. **MESO**, v. 11, p. 241-249, 2009.

KESKINEL, A., AYRES, J. C., AND SYNDER, H.E. Determination of oxidative changes in raw meats by the 2-thiobarbituric acid reactive substances method. **Food Technology**, Chicago, v. 18, p.101-104, 1964.

KIM, Y. H. et al. Role of lactate dehydrogenase in metmyoglobin reduction and color stability of different bovine muscles. **Meat Science**, Barking, v. 83, n. 3, p. 376–382, 2009.

KING, D. A. et al. Contribution of genetic influences to animal-to-animal variation in myoglobin content and beef lean color stability. **Journal of Animal Science**, Champaign, v. 88, n. 3, p. 1160–1167, 2010.

KING, D. A. et al. Influence of animal temperament and stress responsiveness on the carcass quality and beef tenderness of feedlot cattle. **Meat Science**, Barking, v. 74, n. 3, p. 546–556, 2006.

KNOCK, R. C. et al. Effects of potassium lactate, sodium chloride, sodium tripolyphosphate, and sodium acetate on colour, colour stability, and oxidative properties of injection-enhanced beef rib steaks. **Meat Science**, Barking, v. 74, n. 2, p. 312–318, 2006.

KRZYWICKI, K. Assessment of relative content of myoglobin, oxymyoglobin and metmyoglobin at the surface of beef. **Meat Science**, Barking, v. 3, n. 1, p. 1–10, 1979.

LAWRENCE, L. J., FOWLER, V. R., NOVAKOFSKI, J. E. **Growth of Farm Animals**. 3rd Ed. Oxfordshire: CABI, 2012, 352 p.

LAWRIE, R. A. Physiological stress in relation to dark-cutting beef. **Journal of the Science of Food and Agriculture**, London, v. 9, n. 11, p. 721–727, 1958.

LEDWARD, D. A. Post-slaughter influences on the formation of metmyoglobin in beef muscles. **Meat Science**, Barking, v. 15, n. 3, p. 149–171, 1985.

LINDAHL, G. Colour stability of steaks from large beef cuts aged under vacuum or high oxygen modified atmosphere. **Meat Science**, Barking, v. 87, n. 4, p. 428–435, 2011.

LOMIWES, D. et al. Small heat shock proteins and toughness in intermediate pHu beef. **Meat Science**, Barking, v. 95, n. 3, p. 472–479, 2013.

LOMIWES, D. et al. The protection of bovine skeletal myofibrils from proteolytic damage post mortem by small heat shock proteins. **Meat Science**, Barking, v. 97, n. 4, p. 548–557, 2014.

MACDOUGALL, D. B. Changes in the colour and opacity of meat. **Food Chemistry**, London, v. 9, n. 1–2, p. 75–88, 1982.

MACH, N. et al. Association between animal, transportation, slaughterhouse practices, and meat pH in beef. **Meat Science**, Barking, v. 78, p. 232–238, 2008.

MADHAVI, D. L.; CARPENTER, C. E. Aging and Processing Affect Color, Metmyoglobin Reductase and Oxygen Consumption of Beef Muscles. **Journal of Food Science**, Chicago, v. 58, n. 5, p. 939–942, 1993.

MANCINI, R. A.; HUNT, M. C. Current research in meat color. **Meat Science**, Barking, v. 71, n. 1, p. 100–121, 2005.

MANCINI, R. A.; RAMANATHAN, R. Effects of postmortem storage time on color and mitochondria in beef. **Meat Science**, Barking, v. 98, n. 1, p. 65–70, 2014.

MANCINI, R. A.; SEYFERT, M.; HUNT, M. C. Effects of data expression, sample location, and oxygen partial pressure on initial nitric oxide metmyoglobin formation and metmyoglobin-reducing-activity measurement in beef muscle. **Meat Science**, Barking, v. 79, n. 2, p. 244–251, 2008.

MCKEITH, R. O. et al. Mitochondrial abundance and efficiency contribute to lean color of dark cutting beef. **Meat Science**, Barking, v. 116, p. 165–173, 2016.

MCMILLIN, K. W. Where is MAP Going? A review and future potential of modified atmosphere packaging for meat. **Meat Science**, Barking, v. 80, n. 1, p. 43–65, 2008.

MIGUEL, G. Z. et al. Immunocastration improves carcass traits and beef color attributes in Nellore and Nellore-Aberdeen Angus crossbred animals finished in feedlot. **Meat Science**, Barking, v. 96, n. 1, p. 884–891, 2014.

MLYNEK, K.; JANIUK, I.; DZIDO, A. Effect of growth intensity of bulls on the microstructure of musculus longissimus lumborum and meat quality. **Acta Veterinaria Brno**, Brno, v. 81, n. 2, p. 127–131, 2012.

MOORE, M. C., et al. National Beef Quality Audit–2011: In-plant survey of targeted carcass characteristics related to quality, quantity, value, and marketing of fed steers and heifers. **Journal of Animal Science**, Champaign, v. 90, p. 5152–5160, 2012.

O'KEEFFE, M.; HOOD, D. E. Biochemical factors influencing metmyoglobin formation on beef from muscles of differing colour stability. **Meat Science**, Barking, v. 7, n. 3, p. 209–228, 1982.

O'SULLIVAN, M. G.; LE FLOCH, S.; KERRY, J. P. Resting of MAP (modified atmosphere packed) beef steaks prior to cooking and effects on consumer quality. **Meat Science**, Barking, v. 101, p. 13–18, 2015.

OUALI, A. et al. Revisiting the conversion of muscle into meat and the underlying mechanisms. **Meat Science**, Barking, v. 74, n. 1, p. 44–58, 2006.

PAGE, J. K.; WULF, D. M.; SCHWOTZER, T. R. A survey of beef muscle color and pH. **Journal of Animal Science**, Champaign, v. 79, n. 3, p. 678–687, 2001.

PARK, B. Y.; LEE, J. M.; HWANG, I. H. Effect of postmortem metabolic rate on meat color. Asian-Australasian **Journal of Animal Science**, Champaign, v. 20, n. 4, p. 598–604, 2007.

PONNAMPALAM, E. N. et al. Causes and Contributing Factors to "Dark Cutting" Meat: Current Trends and Future Directions: A Review. **Comprehensive Reviews in Food Science and Food Safety**, Chicago, v. 16, p. 400–430, 2017.

POTHAKOS, V. et al. Lactic acid bacteria and their controversial role in fresh meat spoilage. **Meat Science**, Barking, v. 109, p. 66-74, 2015.

PRIOLO, A.; MICOL, D.; AGABRIEL, J. Effects of grass feeding systems on ruminant meat colour and flavour. A review. **Animal Research,** London, v. 50, p. 185–200, 2001.

PULFORD, D. J. et al. The intracellular distribution of small heat shock proteins in post-mortem beef is determined by ultimate pH. **Meat Science**, Barking, v. 79, n. 4, p. 623–630, 2008.

RAMANATHAN, R. et al. Bovine mitochondrial oxygen consumption effects on oxymyoglobin in the presence of lactate as a substrate for respiration. **Meat Science**, Barking, v. 93, n. 4, p. 893–897, 2013.

RAMANATHAN, R. et al. Effects of 4-hydroxy-2-nonenal on beef heart mitochondrial ultrastructure, oxygen consumption, and metmyoglobin reduction. **Meat Science**, Barking, v. 90, n. 3, p. 564–571, 2012.

RAMANATHAN, R. et al. Effects of lactate-enhancement on surface reflectance and absorbance properties of beef longissimus steaks. **Meat Science**, Barking, v. 84, n. 1, p. 219–226, 2010.

REDDY, L. M.; CARPENTER, C. E. Determination of Metmyoglobin Reductase Activity in Bovine Skeletal Muscles. **Journal of Food Science**, Chicago, v. 56, n. 5, p. 1161–1164, 1991.

RENERRE, M. Factors involved in the discoloration of beef meat. **International Journal of Food Science & Technology**, Oxford, v. 25, n. 6, p. 613–630, 1990.

RENERRE, M.; LABAS, R. Biochemical factors influencing metmyoglobin formation in beef muscles. **Meat Science**, Barking, v. 19, n. 2, p. 151–165, 1987.

ROSA, A. et al. Incidence of DFD meat on Brazilian beef cuts. **Meat Science**, Barking, 2016.

SAMMEL, L. M. et al. Comparison of Assays for Metmyoglobin Reducing Ability in Beef Inside and Outside Semimembranosus Muscle. **Journal of Food Science**, Chicago, v. 67, n. 3, p. 978–984, 2002.

SCHEFFLER, T. L., MATARNEH, S. K., ENGLAND, E. M., GERRARD, D. E. Mitochondria influence postmortem metabolism and pH in an in vitro model. **Meat Science**, Barking, v. 110, p. 118–125, 2015

SEIDEMAN, S. C. et al. Utilization of the Intact Male for Red Meat Production : A Review. **Journal of Animal Science**, Champaign, v. 55, n. 4, p. 826–840, 1982.

SEYFERT, M. et al. Color stability, reducing activity, and cytochrome c oxidase activity of five bovine muscles. **Journal of Agricultural and Food Chemistry**, Easton, v. 54, n. 23, p. 8919–8925, 2006.

SHAREDEH, D. et al. Effects of pH and NaCl levels in a beef marinade on physicochemical states of lipids and proteins and on tissue microstructure. **Meat Science**, Barking, v. 110, p. 21-31, 2015.

SMITH G. C. et al. Economic implications of improved color stability in beef. In: DECKER, E. A.; FAUSTMAN, C.; LOPEZ-BOTE, C. J. Antioxidants in muscle foods: nutritional strategies to improve quality. New York: Wiley-Interscience, 2000. chap. 15, p. 397-426.

SØRENSEN, G.; JØRGENSEN, S. S. A critical examination of some experimental variables in the 2-thiobarbituric acid (TBA) test for lipid oxidation in meat products. **Zeitschrift fur Lebensmittel Untersuchung und Forschung**, Berlin, v. 2002, p. 205-210, 1996

STACKHOUSE, R. J. et al. Postrigor citric acid enhancement can alter cooked color but not fresh color of dark-cutting beef. **Journal of Animal Science**, Champaign, v. 94, n. 4, p. 1738–1754, 2016.

SUGAWARA, Y., SHIKAMA, K. Autoxidation of native oxymyoglobin: thermodynamic analysis of the pH profile. **European Journal of Biochemistry**, Berlin, v.110, p. 241–246, 1980.

SUMAN, S. P. et al. Improving beef color stability: Practical strategies and underlying mechanisms. **Meat Science**, Barking, v. 98, n. 3, p. 490–504, 2014.

SUN, X. D.; HOLLEY, R. A. Antimicrobial and Antioxidative Strategies to Reduce Pathogens and Extend the Shelf Life of Fresh Red Meats. **Comprehensive Reviews in Food Science and Food Safety**, Chicago, v. 11, n. 4, p. 340–354, 2012.

TALMANT, A., G. MONIN. Activities of metabolic contractile enzymes in 18 bovine muscles. **Meat Science**, Barking, v. 18, p. 23–40, 1986.

TANG, J. et al. Postmortem oxygen consumption by mitochondria and its effects on myoglobin form and stability. **Journal of Agricultural and Food Chemistry**, Easton, v. 53, n. 4, p. 1223–1230, 2005.

USDA - UNITED STATES DEPARTMENT OF AGRICULTURE. **Livestock and Poultry: World Markets and Trade**, Washington – DC, April, 2017. Retrieved May 15, 2017 from <http://apps.fas.usda.gov/psdonline/circulars/livestock_poultry.pdf>.

VESTERGAARD, M. et al. Influence of feeding intensity, grazing and finishing feeding on meat and eating quality of young bulls and the relationship between muscle fibre characteristics, fibre fragmentation and meat tenderness. **Meat Science**, Barking, v. 54, p. 187–195, 2000.

VILJOEN, H. F.; DE KOCK, H. L.; WEBB, E. C. Consumer acceptability of dark, firm and dry (DFD) and normal pH beef steaks. **Meat Science**, Barking, v. 61, n. 2, p. 181–185, 2002.

VYNCKE, W. Evaluation of direct thiobarbituric acid extraction method for determining oxidative rancidity in mackerel (Scomber scombrus L.). **Fette Seifen Anstrichmittel**, Leinfelden-Echterdingen, v. 77, p. 239-240, 1975

VYNCKE, W. Direct determination of the thiobarbituric acid value in trichloracetic acid extracts of fish as a measure of oxidative rancidity. **Fette Seifen Anstrichmittel**, Leinfelden-Echterdingen, v. 72, p. 1084-1087, 1970.

WĘGLARZ, A. Meat quality defined based on pH and colour depending on cattle category and slaughter season. **Czech Journal of Animal Science**, Praha, v. 55, n. 12, p. 548–556, 2010.

WU, G. et al. Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. **Meat Science**, Barking, v. 98, n. 4, p. 637–645, 2014.

WULF, D. M. et al. Relationships among glycolytic potential, dark cutting (dark, firm, and dry) beef, and cooked beef palatability. **Journal of Animal Science**, Champaign, v. 80, n. 7, p. 1895–1903, 2002.

YOUNG O.A., et al. Effect of cattle diet on some aspect of meat quality, Proc. 43rd ICoMST, Auckland, New Zealand, 1997.