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UNIVERSITÉ BLAISE PASCAL – CLERMONT FERRAND II

École Doctorale des Sciences de la Vie et de la Santé

Tese

CARACTERÍSTICAS BIOQUÍMICAS E ESTRUTURAIS DE MÚSCULOS DE EMA (*Rhea americana*): IMPLICAÇÕES SENSORIAIS E NUTRICIONAIS

RENATA SCHMIDT FILGUERAS

Pelotas, 2010.

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Tese apresentada ao Programa de Pós-Graduação em Ciência e Tecnologia Agroindustrial da Faculdade de Agronomia Eliseu Maciel, Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutor em Ciências: Ciência e Tecnologia Agroindustrial.

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And

UNIVERSITÉ BLAISE PASCAL CLERMONT FERRAND II
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Thesis

Biochemical and structural characterization of rhea *(Rhea americana)* muscles: sensorial and nutritional implications

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«*Un humanisme bien ordonné ne commence pas par soi-même, mais place le monde avant la vie, la vie avant l'homme, le respect des autres êtres avant l'amour-propre.*»

"Um humanismo bem ordenado não começa por si mesmo, mas põe o mundo antes da vida, a vida antes do homem, o respeito pelos outros seres antes do amor próprio."

Claude Lévi-Strauss (*1908-2009*).

To my parents.

RESUMO

As características bioquímicas e a estabilidade oxidativa durante o armazenamento foram estudadas nos músculos *Gastrocnemius pars interna* (GN) e *Iliofiburalis* (IF) de emas *(rhea americana).* Além disso, o estudo histoquímico e morfológico foi conduzido para determinar o tipo metabólico e contráctil das fibras musculares e as diferenças estruturais e ultra-estruturais entre os dois músculos. Por fim, a taxa de digestão e o valor nutricional das proteínas miofibrilares foram investigados após armazenamento/maturação e cocção da carne do músculo GN.

O pH final de ambos os músculos foi semelhante, mas o potencial glicolítico (PG) do músculo IF foi significativamente superior ao do músculo GN. Sob refrigeração (4 °C) e embalagem permeável ao oxigênio os músculos de ema apresentaram diferenças de estabilidade oxidativa. Particularmente, o músculo IF mostrou grande instabilidade de cor e altos níveis de oxidação lipídica e protéica ao final de cinco dias de armazenamento. Sob refrigeração e embalagem a vácuo ambos os músculos foram estáveis durante 28 dias, não apresentando evidências de oxidação. Sob congelamento, enquanto o músculo GN foi estável durante 180 dias, o músculo IF mostrou indícios de oxidação de lipídios e da mioglobina a partir de 90 dias. O perfil dos ácidos graxos, a maior quantidade de lipídios totais e a maior concentração de mioglobina do músculo IF explicam parcialmente sua maior instabilidade oxidativa quando exposto ao oxigênio. Porém, a grande quantidade de glicogênio residual observada no músculo IF após análise bioquímica também parece estar envolvida na ocorrência dos processos oxidativos.

A análise histoquímica dos músculos GN e IF de emas mostrou a presença de somente um tipo de fibra muscular em ambos os músculos, isto é, todas as fibras analisadas foram classificadas como fibras de metabolismo misto (oxidativo e glicolítico) e contração rápida. A homogeneidade do tipo de fibras foi evidenciada após a aplicação de técnicas histoquímicas que determinaram a atividade da m-ATPase, a atividade da succinato desidrogenase (SDH) e presença de glicogênio muscular. Além disso, a observação ultra-estrutural das miofibrilas mostrou áreas de contração e áreas de extensão, assim como abundância de mitocôndrias e de glicogênio, principalmente no músculo IF.

Por fim, o estudo do valor nutricional e da taxa de digestão das proteínas miofibrilares da carne de ema indicaram que a maturação foi menos impactante do que a cocção sobre a oxidação das proteínas e formação de agregados. Após cocção (100 °C, 30 min), a quantidade de agregados aumentou até 400% na carne de ema e os teores de amino ácidos aromáticos caíram. A taxa de digestão pela pepsina diminuiu após tratamento térmico, enquanto a taxa de digestão pela tripsina/quimotripsina manteve-se estável.

Keywords: Rhea americana, qualidade de carnes, oxidação lipídica, oxidação protéica, cor, taxa de digestão, pepsina, tripsina/quimotripsina, tipo de fibras.

ABSTRACT

Biochemical characteristics and oxidative stability during chilling and frozen were studied in *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF) of *rhea americana*. The histochemical and morphometrical study was also conducted to determine fibre types and structural and ultrastructural differences between GN and IF muscles of rhea. Finally, the *in vitro* protein digestibility and the nutritional value of proteins were also investigated after storage/ageing and cooking in GN muscle.

The ultimate pH was similar in both muscles, but the glycolytic potential (GP) was significantly higher in IF than in GN muscle. Under chilling (4 °C) and air-packaging rhea muscles exhibited differences in their stability. In particular, the IF muscle presented high colour instability and high lipid and protein oxidations after 5 days of air-packaged storage. Under chilling (4 °C) and vacuum-packaging both muscles were highly stables during 28 days and did not present evidences of oxidation. Under frozen (-20 °C) GN muscle was perfectly stable during 180 days, but IF muscle presented evidences of lipid and myoglobin oxidation after 90 days of storage. The fatty acid composition, higher lipid content and the higher myoglobin concentration in IF than in GN muscle could partially explain the instability of IF muscle during air-packaging, but the high residual glycogen observed in biochemical analysis also seemed to be involved in the occurrence of the oxidative process.

The histochemical analysis of rhea limb muscles demonstrated the presence of only one type of fibres in both GN and IF muscles, i.e. fast-twitch oxidative and glycolytic (FOG) fibres. The homogeneity of fibres was evident after m-ATPase, SDH and glycogen staining reactions. In addition, the ultrastructural observation of rhea myofibrils showed contracted and stretched areas, as well as abundant glycogen and numerous mitochondria, mainly in IF muscle.

Finally, the study of protein nutritional value and protein rate of digestion indicated that storage/ageing had less impact than cooking on protein oxidation and aggregation. After cooking (100 °C, 30 min) the aggregates increased 400% and the content of aromatic amino acids decreased. The nutritional value of proteins was affected by cooking, as demonstrated by the decrease of pepsin activity rate. However, trypsin chymotrypsin activities were stable after heat treatment.

Keywords: Rhea, muscle, meat quality, meat oxidation, *in vitro* digestibility, fibre type, histology.

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LIST OF PUBLICATIONS AND MANUSCRIPTS

Chapter II. Filgueras, R. S., Gatellier, P., Aubry, L., Thomas, A., Bauchart, D., Durand, D., Zambiazi, R. C., & Santé-Lhoutellier V. (2010). Colour, lipid and protein stability of *Rhea americana* meat during air- and vacuum-packaged storage: Influence of muscle on oxidative processes. *Meat Science,* **accepted**: *<doi 10.1016/j.meat.sci.2010.06.003>*

Chapter III. Filgueras, R. S., Astruc, T., Venien, A., Peyrin, F., Labas, R., Zambiazi, R. C., & Santé-Lhoutellier V. Histological and ultrastructural characterisation of Rhea Americana muscles. Manuscript in preparation to be submitted to *Food Chemistry*.

Chapter IV. Filgueras, R. S., Gatellier, P., Moindrot, C., Zambiazi, R. C., & Santé-Lhoutellier V. Effect of frozen storage duration and cooking on physical and oxidative changes in *M. Gastrocnemius pars interna* and *M. Iliofiburalis* of rhea americana. **Submitted** to *Meat Science.*

Chapter V. Filgueras, R. S., Gatellier, P., Ferreira, C., Zambiazi, R. C., & Santé-Lhoutellier V. Protein digestibility and nutritional value of rhea meat in association with storage and cooking processes. **Submitted** to *Food Chemistry*.

Appendix A. Filgueras, R. S., Gatellier, P., Aubry, L., Ferreira, C. O., Zambiazi, R. C., & Santé-Lhoutellier, V. Impact du mode de conservation sur la couleur et l'acceptabilité de la viande de nandou (*Rhea americana*). Proceedings of *Huitièmes Journées de la Recherche Avicole, St Malo, 25 et 26 mars 2009*. (**Poster**)

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Filgueras R. S., Gatellier P., Moindrot C., Zambiazi R. C., and Santé-lhoutellier V.

Introduction

Consumers have increasing interest for ratites (ostrich, emu and rhea) because their meats have been seen and marketed as a possible alternative to beef supply. Ratite's meat is a red meat and presents low-fat and low-cholesterol contents, as well as appropriated quantities of proteins, minerals and vitamins. Among the commercially ratites used for meat production the rhea market is rather small, and information concerning traits of rhea meat is rare and sparse. A very small number of studies are available on characteristics and stability of processed rhea meat, which greatly difficult industry development, marketing of the meat and, consequently, the acceptance and trust of the consumers.

The overall quality of meat products has been well described in the literature in domestic animals such as ruminants, pigs or poultry and the main factors influencing it have been identified. Indeed, genetic background, rearing environment, slaughter conditions, biochemical changes *post mortem* and fiber type can be considered as key factors for meat quality determinism, but meat qualities include also ethical, sanitary, sensorial, technological and nutritional aspects. In Brazil, the rhea meat production constitutes a source of animal proteins for exportation, but little information on meat quality was available.

The aim of the study was to assess the rhea meat quality of two distinct limb muscles after different conditions of storage and to get deeper knowledge in the determinism of the nutritional quality of rhea meat and in meat in general after processing. The experimental design was conducted 1) to evaluate the oxidative stability of *M. Gastrocnemius pars interna* and *M. Iliofiburalis* during chilling and frozen storage; 2) to establish links between the physicochemical changes *post mortem* and the histochemical/ultrastructural characteristics of both rhea muscles; and 3) to assess the protein rate of digestion and nutritional quality of rhea meat proteins in relation with oxidation, denaturation, and aggregation.

Chapter 1
Literature Review

1.1. RATITE SPECIES

Ratites are flightless or 'running' birds with different anatomical and physiological characteristics when compared to other major bird species. Ratites have no keel on their sternum (*crista sterni*) to anchor their wing muscles, and by consequence, they are unable to fly (Sick, 1997).

There are ten ratite species in the world, including the ostrich (*Struthio camelus*), the emu (*Dromaius novaehollandiae*), two species of rhea (*Rhea americana* and *Pterocnemia pennata*), three species of cassowary (*Casuarius bennetti*, *Casuarius casuarius* and *Casuarius unappendiculatus*), and three species of Kiwi (*Apterix haastii*, *Apteryx owenii* and *Apterix australis*) (Gunski, 1992).

In nature, ratites are generally limited to the southern hemisphere and are essentially omnivores, eating seeds, leaves, fruits, insects, rodents, mollusks, and even small snakes (Toledo & Tavares, 2003). The most current hypothesis for ratite origin is that these birds lived in Gondwana, the continent that included most of the landmasses in today's southern hemisphere, i.e. South America, Africa, Madagascar, Antarctica, India, parts of South Asia and Australia, at 100 million years ago. The separation of the continents at 80 million years ago drove the ostriches to the Old World and the emus and rheas to Australia and America, respectively (Giannoni, 2001).

Ratites have developed the leg muscles and the wings were atrophied. Their ability to run at high speeds is due to their highly specialized pelvic limb musculature. These animals may travel long distances to find food in *sclerophyll* forest (chaparral), savannas and pampas, showing a high adaptability to different climates (Blake, 1996).

Ostrich is the most commercially exploited ratite specie. It has been raised for feather production for thousands of years, but only after the 2nd world war South African's farmers began the commercial exploitation of the meat and leather of ostriches (Huchzermeyer, 1997). Subsequently, the ostrich breeding had a quick expansion in South Africa and many birds were taken to other countries. In the 1990s, the United States comprised the second greater cluster of ostriches and the largest number of researches on ostrich breeding (Giannoni, 2001). As a consequence of this commercial ascension, the interest for other ratite species also increased and emus and rheas started to be explored as a resource for meat, leather and even for oil production (Blake, 1996; Saadoun & Cabrera, 2008).

Due to a belief in the special healthy characteristics of their meats, ratites (ostriches, emus, and rheas) are receiving more and more attention by meat producers in developed markets (Sales & Horbańczuk, 1998). Meat production is primarily under controlled farming and management schemes. Thus, the same factors of diet, age, sex, handling, stress, slaughter practices, *post mortem* ageing and processing that influence properties of meat from domesticated species also can influence ratite meat. The size and economic impact of ostrich industries in South Africa, Australia, and a few other countries compared with the size and impact of the rhea and emu industries has generated more information on ostrich meat compared with the other two ratite meat species.

1.1.1. Rhea

Rhea americana is a native bird from South America (Argentina, Bolivia, Brazil, Paraguay and Uruguay) that belongs at the ratite group (Fig.1.1). There are two species of rhea: the American or greater rhea (*Rhea americana*), and the Darwin's rhea (*Pterocnemia pennata*).

The greater rhea is larger and darker than Darwin's rhea, and it is more widespread, ranging from southeastern of Brazil to the central of Argentina. The *Pterocnemia pennata* is found in the Altiplano and Patagonia regions of South America, differing to *Rhea americana* by presenting plumes on the tarsal segment of the legs (Gunski, 1992).

The taxonomy of rhea is showed below (Gunski, 1992):

Kingdom: Animalia
Phylum: Chordata
Class: Aves
Superorder: Paleognathae
Order: Rheiformes
Family: Rheidae
Genus: Rhea
Species: Rhea americana
Subspecies: - *Rhea a. albescens* (Lynch, Arribalzaga, & Holmberg, 1878);
- *Rhea a. americana* (Linnaeus, 1758);
- *Rhea a. araneipes* (Brodkorb, 1938);
- *Rhea a. intermedia* (Rothschield & Chubb, 1914);
- *Rhea a. nobilis* (Brodkorb, 1939);
Genus: Pterocnemia
Species: Pterocnemia pennata
Subspecies: - *Pterocnemia p. garleppi* (Chub, 1913);
- *Pterocnemia p. tarapacensis* (Chub, 1913);
- *Pterocnemia p. pennata* (D'Orbigny, 1834).

The rhea is also called South American Ostrich in North America, Ema in Brazil, Ñandú in Uruguay, Argentina and Paraguay, and Nandou in France. It is the largest bird found in South America and presents several morphological, genetic and behavioral similarities with emus and ostriches. However, in comparison with these two ratites, rhea is the smallest and presents three toes rather than two as the ostrich.

According to Ontario Ratite Association (ORA, Canada, 1996), the main characteristics of the rhea commercially produced are:

- Adult height: 132 - 165 cm

- Adult weight: 32.2 – 36.7 Kg

- Reproductive period: from May to September (in north hemisphere)

- The average production of eggs: 40 - 50 eggs/year

- First egg laying: at 2 years old

Rheas are omnivorous and prefer to eat broad-leafed plants, but they also eat seeds, roots, fruit, beetles, grasshoppers, lizards, and carrion (Toledo & Tavares, 2003; Gunski, 1992). During the non-breeding season greater rheas may form flocks among 10 and 100 birds, although the Darwin's rhea usually form smaller flocks than this. Rheas are polygamous: each male may court between two and twelve females. After mating, the male builds a nest, in which each female lays her eggs. The nest consists of a simple scrape in the ground, lined with grass and leaves (Sick, 1997).

Figure 1.1. *Rhea americana* – three adult birds.
Source: http://commons.wikimedia.org/wiki/Rhea_americana

1.1.2. Production data

At 17th century the rhea was very abundant in Brazil, Paraguay, Uruguay and, mainly, in Argentina (Guerreiro, 2001). Nevertheless, the number of these birds has seriously decreased in our days, and the specie has almost disappeared from south and southeast of Brazil. The indiscriminate use of wildlife resources is unacceptable and policies have being established for a legal, sustainable and ethical development of the

commercial trade in products from native animal species (Marie, 2006; Mauro, 2002; Mourão, Campos, & Coutinho, 1996). Under legal conditions, in the two last decades, the number of farms developing rhea breeding has increased in South America. No official data about the amount of farms and animals produced were found in the literature, but the main purpose of these farms is to produce meat and products for local markets (Uhart & Milano, 2002). In 1996, it was opened officially an experimental slaughters of rheas in Uruguay. The first experimental slaughter of rhea in Brazil was opened in 2000. A few later, in 2002, Brazilian Ministry of Agriculture (MAPA - Ministério da Agricultura Produção e Abastecimento) recorded the first slaughterhouse for rheas in Southern Brazil.

Besides, it was not only in South America that rhea breeding started to be raised in 1990s. In 1980s, due to economic sanctions imposed by United States to South Africa, the high importation cost of ostrich and its sub-products encouraged North American farmers to invest in other breeding alternatives. Consequently, the commercial interest for South American Ostrich increased significantly in United States and Canada. In 1991 it was established the North American Rhea Association – NARA, and in 1994, over than 15.000 rheas were breeding in United States. At this time, in Canada, the Canadian Rhea Association (CRA) was created and the rhea breeding became also important in this country.

However, as it happened with ostrich and emu industry, rhea industry was built on the belief that consumers would be willing to shift to more nutritious meats and pay a price premium for those meats, but demand in the food industry is also built on other attributes, for example, taste, appearance and price. Acceptance of rhea was complicated by the fact that proper preparation of meat was essential to obtain maximum quality, and methods used for preparing beef were inappropriate for rhea. So, the industry collapses and declines since the last decade.

There is not specific studies about the rhea meat industry emergence and crisis, but according to Turvey & Sparling (2002), the practice of the ratite industry (ostrich, emu and rhea) was based on two different product categories, breeding stock, and end products (meat, oil, and leather). Although they are linked, the perception during the early stages of the industry appeared to be that the breeder market could operate unconstrained by the realities of the end-product markets. Consumer market

requirements are totally different from those of breeder markets; product characteristics and price are important issues, but the ability to guarantee a stable stream of product within tight quality standards and delivery specifications is also essential to secure access to retail chains. The simple infrastructure and marketing channels that were sufficient for the breeder market were completely inadequate for serving retail or wholesale food chains. Processing rhea became a critical issue because conventional lines for other animals were not suitable, but volume was too low to dedicate processing facilities to rhea alone. Small private facilities processed the birds, but there were no grading standards or quality definitions on which customers could rely. So, specific studies on rhea production and meat quality are necessary to sustain the industry and to expand the market of rhea.

1.2. SKELETAL MUSCLE METABOLISM AND CONVERSION OF MUSCLE INTO MEAT

Skeletal muscles are crucial in posture control and locomotion as well as in protecting vital organs. They contain, in addition to muscle fibres, large quantities of supportive connective tissue, a complete vascular supply, and a nerve supply controlling each of the billions of muscle fibres (Fig.1.2.). Also, skeletal muscles serve as storage depots for lipids and contain considerable quantities of extracellular fluids, primarily consisting of water (Kauffman, 2001).

Generally, the conversion of muscle into meat is a complex process in which all mechanisms responsible for the development of meat qualities are very likely interdependent.

1.2.1. Metabolic function of skeletal muscles

Adenosine triphosphate (ATP) is a short term energy supply of cells. It is the major carrier of chemical energy in tissues. In vivo, ATP is maintained at constant level (homeostasis) in cells, but the muscle is also able to perform this task for a while after slaughter (Bendall, 1973). The molecules of adenosine triphosphate (ATP) contain an

array of three linked phosphates (Fig.1.3). The bonds linking the phosphates persist in solution under a variety of conditions, but when they are broken, an unusually large amount of energy is released that can be used to promote a variety of processes (Berg, Tymoczo, & Stryer, 2002).

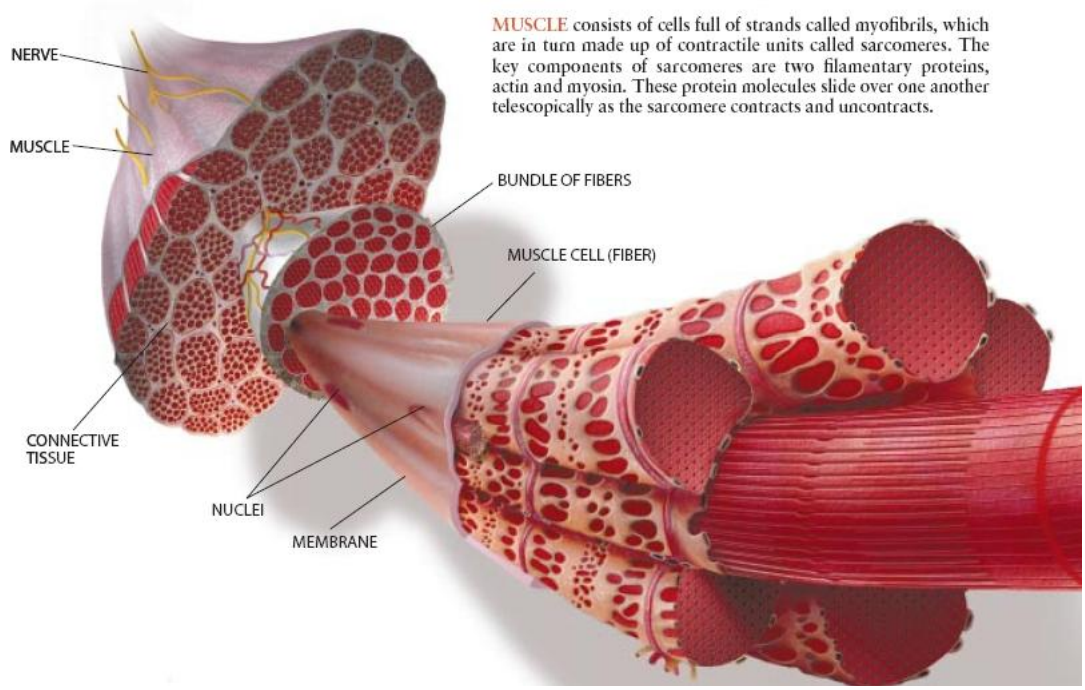

Figure 1.2. Skeletal muscle structure.
Source: SCIENTIFIC AMERICAN, Inc. September 2000, "Muscles, genes and athletic performance".

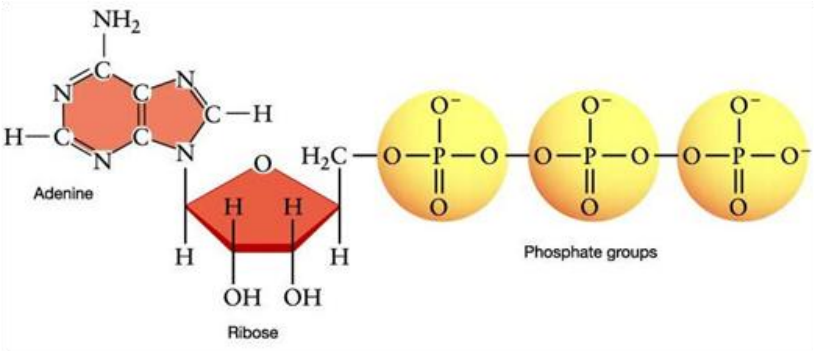

Figure 1.3. The adenosine triphosphate (ATP) molecule.
Source: http://www.griffined.com/pages/biohomepage.html

The most important way for direct synthesis of ATP in organisms is glycolysis, a complex process that renders energy from glucose. Glucose is not only an excellent fuel; it is also a remarkably versatile precursor, capable of supplying a huge array of metabolic intermediates for biosynthetic reactions (Nelson & Cox, 2005).

In animals and vascular plants, glucose has three major destinies: it may be stored (as a polysaccharide), oxidized to a three-carbon compound (pyruvate) via glycolysis to provide ATP and metabolic intermediates, or oxidized via the pentose phosphate (phosphogluconate) pathway to yield ribose 5-phosphate for nucleic acid synthesis and NADPH for reductive biosynthetic processes (Fig.1.4).

Glycolysis is the sequence of reactions that metabolizes one molecule of glucose to two molecules of pyruvate with the concomitant net production of two molecules of ATP (Berg, Tymoczko, & Stryer, 2002; Murray, Granner, Mayer, & Rodwell, 2003; Nelson & Cox, 2005). According to Nelson & Cox (2005), there are 3 possible catabolic fates of the pyruvate formed in glycolysis (Fig. 1.5), but in skeletal muscles only the first and second fates can take place:

1) In aerobic organisms and tissues, under aerobic conditions, glycolysis is the first stage in the complete degradation of glucose. Pyruvate is oxidized, with loss of its carboxyl group as CO_2, to yield the acetyl group of acetyl-coenzyme A. The acetyl group is then oxidized completely to CO_2 by the citric acid cycle. The electrons from these oxidations are passed to O_2 through a chain of carriers in the mitochondrion, to form H_2O. The energy from the electron-transfer reactions drives the synthesis of ATP in the mitochondrion.

2) The second route for pyruvate is its reduction to lactate via lactic acid fermentation. When vigorously contracting skeletal muscle must function under low oxygen conditions (hypoxia), NADH cannot be reoxidized to NAD, but NAD is required as an electron acceptor for further oxidation of pyruvate. Under these conditions pyruvate is reduced to lactate, accepting electrons from NADH and thereby regenerating the NAD necessary for glycolysis to continue.

3) The third major route of pyruvate catabolism leads to ethanol. In some plant tissues and in certain invertebrates, protists, and microorganisms such as brewer's yeast, pyruvate is converted under hypoxic or anaerobic conditions into ethanol and CO_2, a process called ethanol (alcohol) fermentation.

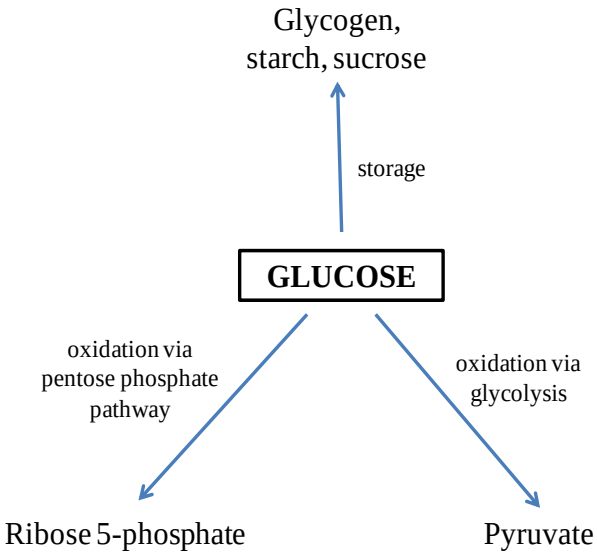

Figure 1.4. Major pathways for glucose utilization.
Source: Nelson & Cox, 2005.

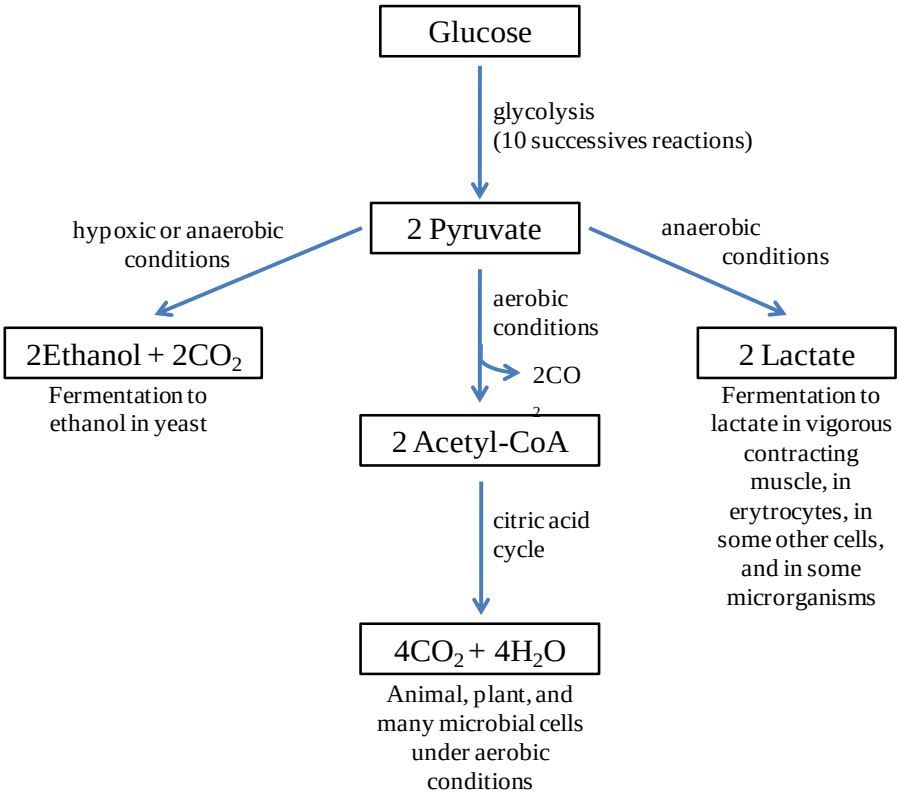

Figure 1.5. Possible catabolic fates of the pyruvate formed.
Source: Nelson & Cox, 2005.

1.2.2. Metabolic changes after slaughter

Immediately after slaughter muscles do not cease to function, but the metabolic functions are markedly altered. The cellular homeostasis mechanism continues to role for some period of time after the decease. The sarcoplasmic reticulum calcium pump and cell membrane sodium-potassium pump persist to function to move their respective ions against the concentration gradients (Greaser, 2001; Lawrie, 1998). Nevertheless, the cessation of blood supply leads to a deprivation of nutrients and oxygen to muscle cells. It reduces the cell oxidation and only reactions following anaerobic pathway persist, essentially the anaerobic glycolysis (Bendall, 1973; Lawrie, 1998). In the exsanguinated animal, glycolysis pathway is the major source for the constant requirement of ATP. Then, the pyruvate generated as a final product of glycolysis is converted to lactic acid, and, as the circulatory system is incapable to remove the metabolites, the lactic acid remains and increases in the muscles. Because there is a fixed supply of glycogen at the time of death, glycolysis can only continue for some limited time period *post mortem*. However, usually glycolysis ceases before all the glycogen is depleted. According to Greaser (2001), the reasons for this cessation are not completely understood, but some possibilities include: (a) the conversion of adenine nucleotides to inosine derivatives may halt the glycolytic flux. Some ATP is regenerated by the myokinase-catalyzed reaction of two moles of ADP to form one mole of ATP and one mole of AMP. The AMP is converted to IMP and ammonia by the enzyme AMP deaminase; and/or (b) the low pH that develops in muscle *post mortem* can inactive one of the glycolytic enzymes. El Rammouz et al. (2004) demonstrated that the activity of AMPdeaminase was positively correlated with ultimate pH in chicken.

Bendall (1973) described the biochemical reactions balance of ATP degradation and synthesis that occurs in the muscle cells immediately after death. The author distinguishes two phases: the *phase of latency* and the *rigor installation phase*.

- The latency phase is characterized by a constant rate of ATP; there is no net consumption of ATP, while phosphocreatine and glycogen concentrations fall down.

The level of ATP can be maintained for some time by a resynthesis from ADP and creatine phosphate (CP).

- The second phase, or the rigor installation phase, is characterized by the ATP disappearance, the AMP deamination and the appearance of NH_3 (NH^{4+}). In this second phase, there is no more phosphocreatine (or only small quantities) and the ATP continues to be regenerated in the muscle cell by the myokinase. When the store of CP is used up, *post mortem* glycolysis can resynthesize ATP, but only ineffectively and the overall level falls.

1.2.3. *Post mortem* pH decline in muscles

The reactions occurring in muscular cells during *post mortem* period are catalysed by the release of calcium ions in the sarcoplasm, which stimulate the ATPase activity of the actomyosin complex. Consequently, there is inorganic phosphate release, lactic acid accumulation and H^+ liberation in the tissue. These phenomena cause a gradual acidification and induce a drop in muscle pH. After death, the muscle acidification continues until the end of the biochemical reaction by anaerobic glycolysis (Fremery & Lineweaver, 1962; McGinnis, Fletche, Papa, & Buhr, 1989).

The fall of pH during the *post mortem* period is characterized by its magnitude and velocity. The rate of the pH fall is determined primarily by the ATPase activity; the amplitude of pH decline depends mainly of muscular glycogen reserves (energy reserves) at the slaughter time (Bendall & Lawrie, 1962).

The main factors affecting the fall of pH and its magnitude and velocity are:

1) Intrinsic factors (linked to the animal): specie, genetic, age, and biological and biochemical characteristics of muscles (fibre type);

2) Extrinsic factors: *ante mortem* stress (fasting, transport, temperature) stunning method, *post mortem* electric stimulation of carcass and chilling conditions.

Concerning the intrinsic factor *biological and biochemical characteristics of muscles*, the amplitude of pH decline (ultimate pH) in muscles *post mortem* is different depending on the type of muscle metabolism (glycolytic and oxidative muscles). This relation will be better commented in the next item of this manuscript (1.3.5. Metabolic and contractile muscle type), but in synthesis:

- Glycolytic muscles have more glycogen in their cells than oxidative muscles. Then, they produce higher amounts of lactic acid than oxidative muscles because they use the glycolytic pathway to produce energy rather than the oxidative pathway. So that, the ultimate pH is generally lesser in muscles presenting more glycolytic fibres than in muscles presenting more oxidative fibres (Hay, Currie, Wolfe, & Sanders, 1973; Laborde, Talmant, & Monin, 1985). However, controversial opinions exist, as reported underneath.

1.2.4. Mechanical changes after slaughter

The conversion of muscle into meat involves the onset of *rigor mortis*, when muscle is acidified and becomes tight, and the ageing, which led to the progressive tenderization of the muscles.

The most important mechanical *post mortem* change is the development of rigor mortis or "stiffness of death". According to Greaser (2001), the time course of rigor mortis is associated to metabolite modifications in muscle cells; the conclusion of rigor corresponds to the point where the ATP has been depleted; the onset period probably starts when the ATP levels starts to decline; and the loss of extensibility is due to the firm attachment of the myosin heads to actin.

In vivo, ATP is necessary to separate myosin heads to actin and allow the filaments to glide over one another. However, without ATP, these two proteins become firmly linked together and no longer allow muscle shortening or extension. The process is rather complicated because not all fibres in a muscle deplete their ATP at the same time. It occurs because there are biological variations and differences in fibre types (Greaser, 2001; Lawrie, 1998).

After the rigor mortis installation, muscles lose irreversibly the property of reduction/extension and the proteins tend to denature. The myofibrillar proteins desmin, troponin T, titin, nebulin, and vinculin all become partially or completely degraded during the first week *post mortem* (Boehm, 1998). Although the proteolytic enzymes responsible for this degradation have not been clearly identified, the patterns of fragments produced and the proteolytic susceptibility in vitro suggest that the calpains are involved. Calpains are calcium-activated proteases originally described by

Dayton et al. (1976). The current hypothesis regarding *post mortem* protein degradation proposes that the calcium in the sarcoplasmic reticulum escapes into the cytosol and activates the calpains after the muscle ATP is depleted (Koohmaraie, 1996). In addition, after death calpastatin activity (the natural inhibitor of the calpains) declines and contributes to the degradation of meat proteins (Greaser, 2001).

1.2.5. Metabolic and contractile muscle types

Muscle fibres are the basic unit for muscle activity, and their traits may explain part of the variation in some meat quality characteristics (Henckel, Oksberg, Erlandsen, Barton-Gade, & Bejerrholm, 1997). However, muscle fibre type composition is highly variable, depending on several factors such as breed, sex, ambient temperature and exercise (Lefaucheur & Gerrard, 1998).

According to their functional and biochemical properties muscle fibres are generally categorized into 3 groups:

- Slow red fibres, rich in myoglobin and with low contraction speed. In these fibres the predominant metabolism for ATP regeneration is oxidative (SO: Slow Twitch Oxidative, βR or I, according to Peter et al. (1972), Ashmore & Doerr (1971), or Brooke & Kaiser (1970) classifications, respectively).

- Red fibres with intermediate characteristics and mixed metabolism (FOG: Fast Twitch Oxidative-Glycolytic, αR, or IIA).

- Fast white fibres, with predominant anaerobic metabolism, deprived in myoglobin and rich in glycolysis enzymes (FG: Fast Twitch Glycolytic, αW or IIB).

Thus, the white fibres (FG, αW or IIB) mediate intense activities that persists very short-time. The intermediates fibres (FOG, αR, or IIA) are suitable for intense activities during long time. And the reds (SO, βR or I) ensure prolonged slow activity (Goldspink & Yang, 1999). The proportions of these types of fibre influence important zootechnical characters, in particular sensorial quality of meat of most species (Picard et al., 1998).

In pigs, the percentage of the different types of fibres in *M. Longissimus dorsi* is approximately 13 % SO, 17 % FOG and 70 % FG. In poultry (chickens and turkeys), the pectoral muscle consists almost exclusively of FG fibres (Lengerken, Maak, & Wicke,

2002) and the *M. Biceps femoris* is composed by 12 % SO, 0.5 % FOG and 87.5 % FG (Papinaho & Fletcher, 1996). The characteristics of the different fibre types are represented in Table 1.1.

The relationship between speed, amplitude of the *post mortem* pH decline and composition of muscle fibres are controversial. According to Xiong et al. (1993) the pH decline in chickens is faster and more pronounced (ultimate pH lower) in white (pectoral muscle) than in red muscles of the thigh. Similarly, Addis (1986) also showed faster pH decline that in white muscle of turkeys. Similarly, in pigs, Klosowska, Klosowski, & Kortz (1975) described more rapid pH decline in white muscles. However, Lefaucheur et al. (1991) have not observed pH difference at 45 minutes *post mortem* in pork *M. Longissimus dorsi* (fast white muscle) and *M. Semispinalis* (red, intermediate). Other results of the same authors indicate a faster pH decline in pork *Psoas major* muscle (intermediate) than in *M. Longissimus dorsi*. According to Lefaucher et al. (1991) this phenomenon may be explained by the fact that muscles rich in slow red fibre, i.e. SO, have a lower ATPase activity, which slows down the speed of the pH decline, but they have also a lower buffer power which would increase the speed of the *post mortem* pH decline (Talmant et al., 1986).

The amplitude of the pH decline (ultimate pH) is dependent on the type of muscle fibres. Indeed, it mainly depends on the rate of muscle glycogen at the slaughter. The white fibres are richer in glycogen than red fibres. Thus, the ultimate pH is lower when glycolytic fibres proportion is higher (Hay, Currie, Wolfe, & Sanders, 1973; Laborde, Talmant, & Monin, 1985).

No information on fibre type of rhea muscles were found in the literature, but according to Rosser & George (1985) the *M. Pectoralis* of ostrich and emu had different proportions of FG, FOG, and SO depending on the portion of the muscle (cranial, central and caudal portions), with FTG fibres predominating in the *M. Pectoralis* from male and female ostriches, while emus had somewhat homogenous proportions of SO, FOG, and FG fibres.

Although the fibre types were not easily distinguished and appeared continuously variable in emu muscles, muscles with prevalence of white fibres presented small intermediate fast oxidative-glycolytic (FOG) fibres and large fast glycolytic (FG) fibres, while muscles presenting mixed kinds of fibres presented large FG

and FOG fibres, and small SO fibres. The *Gastrocnemius lateralis*, *G. intermedius caudalis* and *G. intermedius medialis* muscles had 55 to 58% FG and 42 to 45% FOG (Patak & Baldwin, 1993).

Histochemical analyses showed the presence of FG, FOG, and SO only in *M. Gastrocnemius pars externa* of ostriches; the FG fibres were absent in *M. Tibialis cranialis caput femorale, M. Tibialis cranialis caput tibiale,* and *M. Fibularis longus* (Velotto & Crasto, 2004). There were more SO than FOG fibres in both *M. Tibialis cranialis*. The FG fibres outnumbered the other fibres, followed by the SO and FOG fibres in the *M. Gastrocnemius pars externa* (Velotto & Crasto, 2004).

Table 1.1

Muscle type fibre characteristics.

	Muscle type fibers		
	SO	***FOG***	***FG***
Color	red	pale red	white
Section diameter	small	intermediate	large
Resistance to fatigue	high	intermediate	low
Activity used for	aerobic	long-term anaerobic	short-term anaerobic
Force production	low	high	low
Capillary density	high	intermediate	low
Mitochondrial density	high	high	low
Mioglobin level	high	intermediate	low
Oxidative capacity	high	high	low
Glycolytic capacity	low	high	high
Energetic metabolism	oxidative	oxidative/glycolytic	glycolytic

Source: Judge *et al.*, 1989; and Lengerken *et al.*, 2002.

1.3. MEAT COLOUR

According to Young & West (2001), the colour of an object is the perception of the spatial patterns of different wavelengths of light that emanate from that object. The fundamental colour of an object, whether shiny, matte or translucent, depends on the absorbance of light by both the pigments and the object. In meats, fresh colour is the most important factor influencing consumer purchasing decision because consumers associate colour as indicator of freshness and wholesome. However, the pigment stability in meat is very variable and influenced by various factors.

1.3.1. Meat pigments

The colour of ratite's meat is red and the predominant pigment responsible for most of this colour is the sarcoplasmic protein called myoglobin. This protein is a globular protein containing a porphyrin ring structure and about 153 known amino acids (Fig. 1.6). Two histidine residues are particularly essential to myoglobin structure and function, as well as muscle-food colour stability: the distal histidine (H64) and the proximal histidine (H93) (Mancini, 2009). Centrally located within this globular single-chain protein is the iron, which plays a vital role in the visible colour transformations that occur on the surface of meat products (Fig. 1.7.). The combination of iron and porphyrin is called heme.

Myoglobin is chemically very similar to the blood protein hemoglobin, which also contains iron bounded in porphyrin. The muscle contains also cytochrome and in a lesser extent hemoglobin, but their content is much lower than that present in the myoglobin. The appearance of meat products is influenced not only by myoglobin, but also by the bone marrow and fat discoloration. However, because myoglobin is the major pigment in muscle, measurements of iron concentration, myoglobin concentration and colour are all strongly correlated (Young & West, 2001).

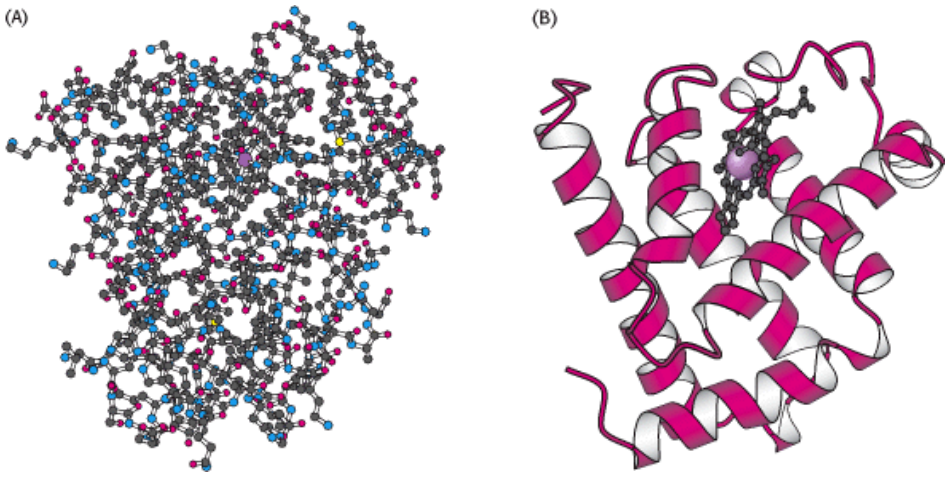

Figure 1.6. Three-dimensional structure of myoglobin. (A) This ball-and-stick model shows all nonhydrogen atoms and reveals many interactions between the amino acids. (B) A schematic view shows that the protein consists largely of helices. The heme group is shown in black and the iron atom is shown as a purple sphere.
Source: Berg, Tymoczko, & Stryer, 2002.

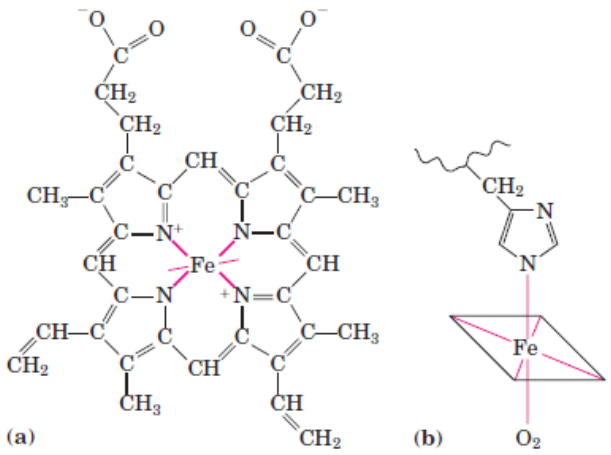

Figure 1.7. The heme group; (a) Organic ring structure, protoporphyrin, to which is bound an iron atom in its ferrous (Fe2) state. (b) Perpendicular coordination bonds is bound to a nitrogen atom of a histidine residue. The other is "open" and serves as the binding site for an O_2 molecule.
Source: Nelson & Cox, 2005.

1.3.2. Myoglobin chemistry

The porphyrin ring structure held in the confines of the myoglobin protein accounts for four of the six coordination sites available on the iron atom (Fig. 1.7). These four sites are the nitrogen atoms of the porphyrin's pyrrole groups. A fifth coordination site is the strategically placed histidine molecule (H93) that is resident in the globular protein which, in addition to hydrophobic interactions, links the prosthetic group to apoprotein. The sixth coordination site is available for binding oxygen or other small molecule.

As reviewed by Mancini & Hunt (2005) and Mancini (2009) and shows in figure 1.8., the major chemical forms of myoglobin that is primarily responsible for meat colour are:

1) ***Deoxymyoglobin:*** Results from a combination of an unoccupied 6^{th} coordination site and ferrous heme iron (Fe^{2+}). It results in the purplish-red or purplish-pink colour that is typically associated with meats that are not exposed to oxygen (vacuum-packaged products) and/or muscles immediately after cutting. Very low oxygen tension (<1.4 mm Hg) is required to maintain myoglobin in a deoxygenated state.

2) ***Oxymyoglobin:*** Occurs when myoglobin is exposed to oxygen and is characterized by the development of a bright cherry-red colour. No change in iron's valence occurs during oxygenation although the 6^{th} coordination site is occupied by diatomic oxygen. As exposure to oxygen increases, the oxymyoglobin penetrates deeper beneath the meat's surface. Depth of oxygen penetration and thickness of the oxymyoglobin layer depends on the meat's temperature, oxygen partial pressure, pH, and competition for oxygen by other respiratory processes.

3) ***Metmyoglobin:*** It results from the oxidation of the heme iron within either deoxy- or oxymyoglobin. The brown colour of this layer results from low oxygen partial pressure, which promotes myoglobin oxidation. The discoloration rate depends on numerous factors including the oxygen partial pressure, temperature, pH, meat's reducing activity, and in some cases, the microbial growth.

4) ***Metmyoglobin reduction:*** Reduction of metmyoglobin is crucial to meat colour life and greatly depends on muscle's oxygen scavenging enzymes, reducing enzyme systems, and the NADH pool, which is limited in *post mortem* muscle.

Oxymyoglobin is not converted directly to deoxymyoglobin, but first proceeds through the ferric redox state at low-oxygen partial pressures. Endogenous oxygen removal to achieve low-oxygen partial pressure occurs via oxygen consumption, which likely results in oxidation of oxy- to metmyoglobin. However, this process is often troublesome because subsequent deoxymyoglobin formation depends on the muscles reducing capacity plus further reduction in oxygen tension. For example, chemical reduction of oxymyoglobin poses a problem when packaging bloomed product in vacuum or ultra-low-oxygen atmospheres because the meat chemistry may not be capable of further oxygen consumption coupled with reduction of ferric to ferrous iron (i.e. reaction three cannot be completed) (Mancini & Hunt, 2005).

5) ***Carboxymyoglobin:*** When carbon monoxide binds strongly to myoglobin it forms a cherry-red pigment called carboxymyoglobin. Exactly how the myoglobin derivatives can form carboxymyoglobin is unclear. Carbon monoxide can bind to the vacant 6^{th} position of deoxymyoglobin and form a very bright-red colour that is relatively stable. It appears that deoxymyoglobin is more readily converted to carboxymyoglobin than is oxy- or metmyoglobin. Nevertheless, carbon monoxide will slowly dissociate from myoglobin after carboxymyoglobin is exposed to atmospheres free of carbon monoxide.

In animal tissue, the muscles often have different content of myoglobin, generally reflecting their physiological role in the animal. Muscles involved in sustained repetitive action, like breathing (diaphragm), contain higher concentrations of myoglobin than muscles not used in a continue way. The lateral line muscle of fish, which is used for sustain low-power movement, is very much redder than the muscle groups used for short bursts of high-power movement. The *Pectoralis* muscle of chicken, used for power takeoffs, is paler than leg muscles (Young et al., 2001).

Muscles with low amount of myoglobin are generally adapted to glycolysis as a means of generating ATP, whereas muscles rich in myoglobin generate ATP through lipid oxidative metabolism. At the same way that the redder muscles are richer in mitochondria, as part of the oxidative machinery, they are also richer in cytochromes. As the name suggests, cytochromes are colored, again due to iron bound in porphyrin. However, cytochromes are present in much lower concentrations than myoglobin

(Renerre & Labas, 1984; Young et al., 2001). Thus, the colour of meat is dominated by the colour reactions of the myoglobin (Fig. 1.8).

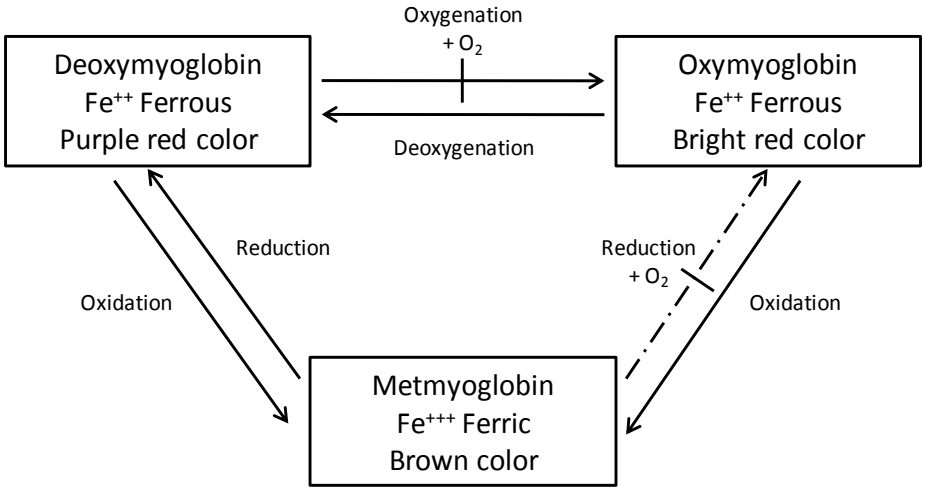

Figure 1.8. Oxido-reduction reactions in myoglobin.

1.5.3. Measurement of colour

Meat colour is measured for many reasons, including grading, matching customer specifications, measuring consumer response to colour, measuring colour changes, and determining the causes of discoloration. The measurement of meat colour demands a systematic approach to data collection, whether colour is scored by a sensory panel (subjective assessment) or measured by an instrument (objective assessment).

In visual colour evaluation, data obtained from hedonic scales is a standard which consumer satisfaction should be based. Although consumer panel are not suitable for describing meat colour, they are ideal for evaluating acceptance and satisfaction (Mancini, 2009). Any of techniques used to assess visual meat colour are complicated by the heterogeneity of meat and the environment in which the assessment are carried out. For instance, panelists scoring the colour acceptability of lean meat can be unconsciously influenced by the colour of the associated fat. Recommendations for visual appraisal of meat are given by the American Meat Science Association (AMSA, 1991)

Objective (instrumental) colour evaluations are based on three-dimensional "colour space" parameters and are usually performed with reflectance colorimeters. A reference method for meat colour measurement (Honikel, 1998) specifies the L*, a*, b* colour scale. L* is a measure of lightness where 0 equals black and 100 equals white. Values of a* indicate redness and b* values indicate yellowness. Hue angle, which defines the colour, is arctangent (b*/a*) determined by rotation about the a* and b* axes. Chroma, a measure of colour intensity, is calculated as $\sqrt{(a^{*2} + b^{*2})}$.

Several methods are available to estimate the amount of each myoglobin redox form on the surface of meat (AMSA, 1991). Since colour research often requires non-invasive, repeatable, and rapid stimulation of surface myoglobin redox state, most quantification methodology is based on reflectance spectrophotometry and isobestic wavelengths (wavelength at which reflectance is equal for two or more redox forms).

Although not a perfect isobestic point, 525 nm has been used for all three pigments, and 572 nm for the reduced (Fe^{2+}) pigments (Fig. 1.9). Stewart et al. (1965), Van den Oord & Wesdorp (1971), and Krzywicki (1979) have each devised equations that yield values on proportions of the three major myoglobin pigments. These equations can be applied to red meat such as beef and ratites, but it is less obvious for "white meat".

Instrumental data must ultimately be correlated with visual judgments. Colour measurements indicating the proportion of meat pigments, usually metmyoglobin, have often been correlated with analytical panel and consumer scores. Colour space parameters have been correlated with all manner of subjective scales. Scales used to grade meat often relate to the paleness or darkness of meat or to chroma. Several studies of vitamin E effects on discoloration of beef during retail display indicate that a* values and hue angle are good indicators of discoloration (Lauzurica, Fuente, Díaz, Alvarez, Perez, & Cañeque, 2005). As discoloration progresses, a* values fall and hue angles increase; as well, colour intensity (chroma) falls, and the meat becomes fade.

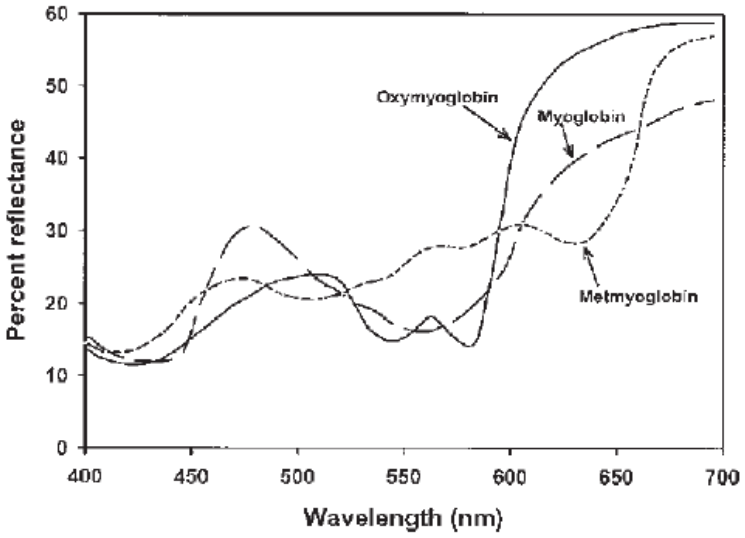

Figure 1.9. Comparative reflectance spectra for solutions of myoglobin (deoxymyoglobin), oxymyoglobin, and metmyoglobin.
Source: Hui, Nip, Rogers, & Young, 2001.

1.5.4. Ratite's meat colour

The colour of raw ostrich *M. Flexor cruris* and *M. Iliofiburalis* has been studied by Paleari, Camisasca, Beretta, Renon, Corsico, Bertolo, & Crivelli (1998). They reported similar L* values to beef and darker than turkey thigh meat. Moreover yellowness b*, in ostrich was generally higher than for beef or turkey and the cooked ostrich meat was darker than turkey or beef.

Few studies have been conducted considering production factors influencing meat colour. The colour reflectance decreased and a* and b* increased with advanced age at slaughter (Hoffman & Fisher, 2001). Pre-slaughter electrolyte treatment of ostriches did not affect L*, a* and b* values of drum and thigh ostrich meat (Schaefer, Robertson, & Brereton, 1997). But conversion of muscle into meat and storage affects the colour coordinates. Indeed, lightness (L*) and yellowness (b*) value increased until 4.5 to 9 hours *post mortem*, followed by a decrease and then a increase through 5 days storage (Thomas, Gondoza, Hoffman, Oosthuizen, & Naudé, 2004).

The heme pigment can be defined as a pro-oxidant substance because its oxidation has been described to be a major catalyst of free radical formation such as superoxide radical and peroxyl radical. In ostrich meat, the heme pigment content was lower in the *M. Gastrocnemius pars interna, M. Iliofiburalis* and *M. Femorotibialis medius*

than in the *M. ambient, M. Iliotibialis lateralis* and *M. Iliofemoralis* (Sales, 1996). According to Ramos et al. (2009), the total iron of rhea meat showed a higher content in *Iliotibialis lateralis* than in *Obturatorius medialis* and *Iliofiburalis* muscles, but the level of heme iron was different between these three rhea muscles. The rhea *M. Iliotibialis lateralis* showed the highest level followed by the *Obturatorius medialis* and *Iliofiburalis* muscles. Rhea had a high level of total iron and heme iron in comparison to ostrich, chicken, beef, lamb and pork (Lombardi-Boccia, Martínez-Domínguez, Aguzzi, & Rincón-León, 2002; Ramos, Cabrera, del Puerto, & Saadoun, 2009).

Iron pigment in the *M. Iliofiburalis* and *M. Iliotibialis lateralis* was lower than in *M. Gastrocnemius medialis*, and *M. Fiburalis longus* of emu at slaughter ages of 6 to more than 20 months (Berge, Lepetit, Renerre, & Touraille, 1997). Variations in lightness (L*) between different emu muscles were small, while the colour stability after air-permeable and vacuum-packaging was more variable between muscles (Berge et al., 1997).

1.4. MEAT STORAGE AND PRESERVATION

1.4.1. Fresh meat

The methods used in meat preservation aim to inhibit microbial spoilage, and to minimize the depreciation of the quality of the product. The extent of depreciation of the quality is largely determined by the time of storage (Lawrie, 1998). The quality of meat is judged by its bacterial state and appearance. Bacterial state is subjectively assessed by the presence or absence of odour or slime, but quantitative measurements can be carried out to determine the total viable counts of microorganisms and the presence of specific pathogens or indicator organisms. Appearance criteria are primarily; colour, percentage of fat and lean and amount of drip Most of red meat are sold as fresh meat at an unprocessed state. These meats are very perishable raw materials, the storage life at low temperature extend the shelf life to few days (James, 2000).

1.4.2. Frozen meat

Freezing is a widely accepted preservation method used to store meat for relatively long periods of time. Utilizing frozen products rather than chilled offers the advantages of increased storage time, greater flexibility in inventory and greater product control (Pietrasik & Janz, 2009). However, freezing and frozen storage of meat can affect the structural and chemical properties of muscle foods, and influence meat quality attributes such as thawing loss, colour and tenderness (Farouk & Swan, 1998; Honikel, Kim, Hamm, & Roncales, 1986). It is commonly known that, during the freezing process, intracellular juice is expelled by osmosis to the extracellular space forming ice crystals that later cause juice loss from meat during thawing (Farouk & Swan, 1998). These types of effects can strongly influence the quality attributes of meat and meat products and consumer acceptance (Pietrasik & Janz, 2009). Packaging is another important factor affecting the shelf-life of meats. Large, wholesale meat cuts are often vacuum sealed to prevent lipid oxidation and formation of metmyoglobin. For retail meats, cooked or raw, films with low water permeability and adequate mechanical strength are required. To prevent the products from being exposed to light, thereby eliminating oxidation catalyzed by light-sensitive compounds, nontransparent outside cardboard-type packages are commonly used (Bell, 2001).

1.4.3. Preservation methods

For meat and most of fresh products, the major cause of deterioration is microbial spoilage, but other causes of product deterioration include desiccation, colour change, and oxidative rancidity. To achieve the preservative function, packaging must restrict microbial growth, prevent moisture loss, and control gaseous exchange between the package and ambient atmospheres (Bell, 2001).

Loss of water from fresh meat reduces the weight of meat available for sale and in extreme cases renders the product unmarketable due its unattractive appearance. However, enclosing meat within a water-impermeable film, while preventing moisture loss, will accelerate the onset of microbial growth because water activity remains high.

As reviewed previously in this manuscript (1.5.2. Myoglobin chemistry), the colour of raw meats is determined by the oxidation state of myoglobin. When oxygen is

absent, myoglobin is in deoxymyoglobin form, which gives meat in vacuum-packaging the purple-red colour. Oxygenation of myoglobin (oxymyoglobin) leads to a bright red colour that consumers associate with freshness. The deoxymyoglobin/oxymyoglobin reaction is reversible and occurs in response to changes in the partial pressure of oxygen surrounding the meat (Mancini & Hunt, 2005). Oxidized myoglobin (metmyoglobin) is brown, a colour associated with the loss of meat quality. Metmyoglobin forms most rapidly at low oxygen concentrations, around 0.5%. As a result, either a highly aerobic or a completely anoxic environment is required to prevent browning. However, as the consumer associates a bright red meat colour with freshness, the packaging requirements for retail display and extended storage are unlikely to be the same (Bell, 2001; Mancini, 2009).

In addition, the reaction of fats with oxygen is responsible for the development of rancid odors and flavors. Oxidation takes place with atmospheric oxygen and is often accelerated by heat, light, high energy radiation, and various oxidation catalysts. In meats, the development of rancidity results primarily from a free radical chain reaction. This means that an anoxic packaging environment may slow but will not prevent the development of rancidity if product is packaged after the initial oxygen–fat reaction has occurred (Bell, 2001).

- ***Non preservative packaging***

Non preservative packaging surrounds and protects the meat from contamination and water loss, but it does not create package conditions very different from ambient ones. Consequently, unless microbial growth can be prevented by freezing or retarded by chilling, product in this kind of packs is highly perishable, and has a short shelf-life. Wrappers are the simplest type of flexible package, in which a sheet material is used to enclose a quantity of product. Examples of this type of packaging relevant to meat include greaseproof paper and plastic films permeable to oxygen. Such packages are generally not sealed by plastic welding.

- ***Preservative packaging***

This group of packaging is characterized by an ability to extend product life by modifying or restricting microbial growth. This is achieved by creating and maintaining conditions in the packs that differ from those of the ambient environment.

In vacuum packaging product is placed into a bag or pouch that is evacuated and heat sealed. The packaging material must have a low permeability to oxygen so that the anoxic in-pack environment is maintained. Vacuum-packaging is widely used as a primary transport and storage packaging for cuts. However, its use at retail has been limited because of the unattractive presentation of product: a purple-red colour due to deoxymyoglobin, squashed appearance, and drip accumulation. Vacuum-packaging is widely used for sliced processed meats, for which the colour is produced by the nitrite cure and is protected by anoxia. Moreover, the rigidity and superior water binding of processed meats mitigate deformation and drip problems (Bell, 2001).

1.5. MEAT OXIDATION

Oxidants are reactive oxygen species (ROS) which have the ability, directly or indirectly, to damage biomolecules, including lipids, proteins, DNA, and carbohydrates. ROS such as the superoxide anion (O_2), hydrogen peroxide (H_2O_2), and the hydroxyl radical (OH•) are generated under numerous conditions *in vivo* (Shacter, 2000).

1.5.1. Lipid oxidation

Lipids are present in muscles as structural components of membranes, as storage droplets of triacylglycerol within muscle fibres, and as adipose tissue (marbling fat). Lipid oxidation is one of the main factors limiting the quality and acceptability of meats and muscle foods (Renerre, 2000, Morrissey et al., 1998). It leads to discoloration, drip losses, off-odor and off-flavor development, texture defects and the production of potentially toxic compounds (Richards et al., 2002; Morrissey et al., 1998). Oxidation of lipids is a chain reaction that consists of initiation, propagation, and termination reactions, and involves the production of free radicals (Renerre, 2000), as shown below:

1. Initiation:

(a) $RH + O_2 \rightarrow R\bullet + \bullet OOH$

2. Propagation:

(b) $R\bullet + O_2 \rightarrow ROO\bullet$

(c) $RH + ROO\bullet \rightarrow ROOH + R\bullet$

(d) $ROOH \rightarrow RO\bullet + \bullet OH$

3. Termination:

(e) $R\bullet + R\bullet \rightarrow R{-}R$

(f) $R\bullet + ROO\bullet \rightarrow ROOR$

(g) $ROO\bullet + ROO\bullet \rightarrow ROOR + O_2$

The initiation of lipid oxidation in meat products is generally believed to occur at the membrane level in the polyunsaturated phospholipids fraction (Gray, Gomaa, & Buckley, 1996). The initiators participating in this reaction can be heat, metals or light. The final termination step gives rise to a broad range of secondary oxidation products such as aldehydes, alkanes, conjugated diens and various other oxidation products. Aldehydes, alcohols, unsaturated hydrocarbons (hexanal) and other oxidation products can make the flavour and shelf life of meat unacceptable (Gray et al., 1996), can modify membrane structure (Monahan et al., 1994), and can also be regarded as a potential health risk (Guardiola, Codony, Addis, Rafecas, & Boatella, 1996).

Lipid oxidation can be influenced by both intrinsic and extrinsic factors such as the concentration of pro-oxidants, endogenous ferrous iron, myoglobin, enzymes, pH, temperature, ionic strength, oxygen consumption reaction and the fatty acid composition of the meat (Andreo, Doval, Romero, & Judis, 2003; Renerre & Labas, 1987). Meats containing high concentration of polyunsaturated fatty acids are more susceptible to oxidation. Furthermore, the concentration of ferrous iron and its ability be active in the lipid oxidation reaction is a key factor causing differences among species and cuts of meat. Other constituents of meat including enzymatic and non-enzymatic reducing

systems can accelerate oxidation by converting iron from the inactive ferric form to the active ferrous state (Foegeding et al., 1996).

1.5.2. Lipid oxidation x Myoglobin oxidation

Lipid oxidation and myoglobin oxidation in meat are coupled and both reactions appear capable of influencing each other. The oxidation of oxymyoglobin results in the production of metmyoglobin and H_2O_2 necessary to induce lipid oxidation. On the other hand, aldehyde formed by lipid oxidation alter myoglobin redox stability, resulting in the promoted oxidation of oxymyoglobin and the formation of adduct with myoglobin through covalent modification (Michael adducts).

Heme pigments, especially myoglobin, catalyze the lipid oxidation in meat (Love, 1983), operating as pro-oxidant. Morey, Hansen and Brown (1973) found that H_2O_2 acting as an oxidizing agent caused changes in the oxidation state of the iron in myoglobin, and induced the formation of red-brown color. The interaction of H_2O_2 with metmyoglobin led very rapidly to generation of active species, which could initiate lipid peroxidation (Chan, Faustman, Yin, & Decker, 1997; Faustman, Liebler, McClure, & Sun, 1999).

On the other hand, aldehydes produced during lipid oxidation can form adducts with proteins and this may have an impact on protein stability and functionality as well as on the color stability of meat (Lynch & Faustman, 2000). Lynch & Faustman (2000) determined the effect of aldehyde lipid oxidation products on oxymyoglobin oxidation, metmyoglobin reduction and the catalytic activity of metmyoglobin as a lipid pro-oxidant in vitro. The covalent attachment of aldehydes to oxymyoglobin rendered oxymyoglobin more susceptible to oxidation (Faustman, Liebler, McClure, & Sun, 1999). The covalent binding of α, β-unsaturated aldehydes to oxymyoglobin at key amino acid residues may subsequently lead to alter tertiary structure of the protein and increases susceptibility to oxidation. This would result in a loss of physiological activity and the brown discoloration in fresh meat (Alderton, Faustman, Liebler, & Hill, 2003).

1.5.3. Protein oxidation

Interactions between lipids and proteins have a significant effect on the progress of oxidative reactions. Under strong interactions, the oxidation reactions can easily transfer from lipids to proteins. Oxidation reactions affect the quality of meat, but they also have an impact on the charge and conformation of the protein three-dimensional structure (exposure of hydrophobic groups, changes in secondary structure and disulfide groups), loss of enzyme activity, and changes in the nutritional value (loss of essential amino acids) (Kamin-Belsky, Brillon, Arav, & Shaklai, 1996; Liu & Xiong, 2000; Santé-Lhoutellier, Astruc, Marinova, Grève, & Gatellier, 2008). In addition, the modified proteins will have different functional properties from those of their unmodified molecules; their emulsifying, foaming, gelling, and water binding properties may be affected as well as the texture of food will be changed (Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004a,b).

Oxidation of the proteins and amino acids is affected by many environmental factors such as pH, temperature, water activity, and the presence of catalysts or inhibitors, and three-dimensional structures of each protein affect how proteins can interact with lipids. The most sensitive amino acids toward oxidation are heterocyclic amino acids. Amino and phenolic groups are more susceptible to oxidation. Due to their structure, tryptophan, histidine, and proline, but also lysine, cysteine, methionine, and tyrosine (Fig.1.10) are prone to oxidation where the hydrogen atom is abstracted either from OH-, S, or N-containing groups (Berlett & Stadtman, 1997; Stadtman, 2004).

Due to the oxidation, methionine is transformed into methionine sulfone, tryptophan into kynurenine, and tyrosine into dityrosine (Stadtman, 2004). Oxidative cleavage of the peptide main chain and the oxidation of the side chains of lysine, proline, arginine, and threonine have been shown to yield carbonyl derivatives (Berlett & Stadtman, 1997).

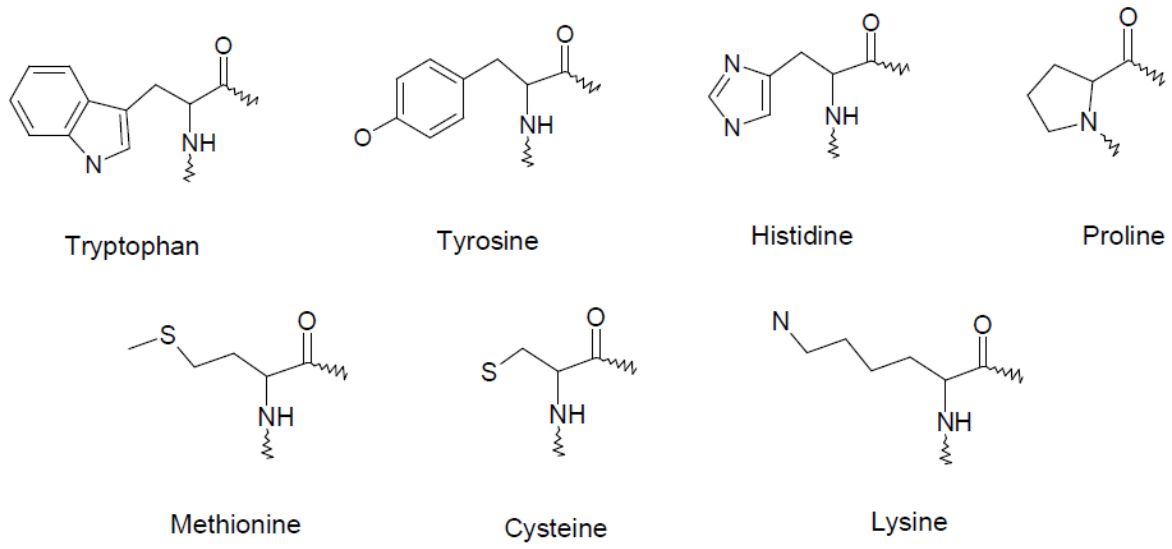

Figure 1.10. Structure of amino acid residues prone to oxidation.
Source: Viljanen, 2005.

Primary lipid oxidation products (hydroperoxides) and secondary lipid oxidation products (aldehydes and ketones) can react with proteins, and cause protein oxidation. Protein oxidation occurs via free-radical reactions in which peroxyl radicals (ROO•) formed during lipid oxidation can abstract hydrogen atoms from protein molecules (PH) (Reaction 1). Consequently, protein radicals are formed (P•), and they can in turn create a protein net (P-P) due to the cross-linking (Reaction 2).

$$ROO\bullet + PH \rightarrow P\bullet + ROOH \qquad \text{Reaction 1}$$

$$P\bullet + P\bullet \rightarrow P\text{-}P \text{ (protein net)} \qquad \text{Reaction 2}$$

It is also postulated that the protein oxidation process can occur via non-covalent complex formation by both electrostatic and hydrophobic attractions between lipid hydroperoxide (ROOH) or secondary lipid oxidation products (mainly aldehydes and ketones, breakdown products of lipid hydroperoxides), and the nitrogen or sulfur centers of reactive amino acid residues of the protein (PH) (Reaction 3) (Berlett & Stadtman, 1997).

$$ROOH + PH \rightarrow [ROOH\text{---}HP] \rightarrow RO\bullet + P\bullet + H2O \qquad \text{Reaction 3}$$

Oxidation of proteins by lipid oxidation products can furthermore lead to the oxidation of amino acid residue side chains, cleavage of the peptide bonds, and formation of the covalent protein-protein cross-linked derivatives (Berlett & Stadtman, 1997). Oxidative cleavage of the peptide bond in the main chain leads to the formation of peptide fragments and the oxidation of the side chains of lysine, proline, tryptophan, arginine, and threonine, yielding to protein-carbonyl compounds.

The reaction between amino acids and secondary lipid oxidation products leads to formation of carbonyl groups in the proteins (Stadtman, 2004). In addition, the lipid oxidation products (hydroperoxides or aldehydes) may react with free amino groups of the protein to generate fluorescent compounds and promote polymerization. Besides, proteins can be cross-linked by carbonyl-protein cross-linking reactions followed by a pyrrole polymerization, derived from interaction between the lipid oxidation products and proteins amino acid residues (Zhu, Spink, Yan, Bank, & DeCaprio, 1994; Hidalgo & Zamora, 1993; Zamora, Alaiz, & Hidalgo, 2000).

The hydrophobic environment of proteins has been shown to decrease due to the protein lipid interaction leading to the modification of the environment of aromatic amino acids (Gatellier, Kondjoyan, Portaguen, Grève, Yoon, Santé-Lhoutellier, 2009; Santé-Lhoutellier et al., 2008).

1.5.4. Formation of the fluorescent protein-lipid complexes

The protein-lipid complexes due to the protein-lipid interaction are formed rapidly. Especially lysine residues (contains free amino group) in the proteins can form strong fluoresce complexes with aldehydes resulting in polymerized products typical for non-enzymatic browning (Berlett & Stadtman, 1997; Stadtman, 2004). If protein amino compounds are involved as amino donors, fluorescent substances formed would remain attached to the amino constituent (protein). Intramolecular and intermolecular cross-linking may also occur. In addition, dityrosine, the oxidized form of tyrosine residues, can fluorescent at the similar excitation and emission wavelengths (Ex ~350nm, Em ~430nm) (Kikugawa, Kato, & Hayasaka, 1991; Viljanen, 2005) (Fig. 1.11).

1.5.5. Formation of Schiff bases and Michael adducts

Secondary lipid oxidation products, such as aldehydes, react mainly with amino acids via condensation reaction to form Schiff's bases and by Michael adduct reactions. The reaction occurs between carbon atom of the aldehyde (either from lipid or sugar) and the nucleophilic amino acid residues (the e-amino group of lysine, the imidazole moiety of histidine or the sulfhydryls group of cysteine) (Uchida & Stadtman, 1993; Stadtman & Berlett, 1997).

The formed Schiff bases or Michael adducts may subsequently participate in the formation of both intra- and intermolecular cross-links with other amino acid residues present such as other lysine, cysteine, or histidine residues (Liu et al., 2003) (Fig. 1.12). The formed cross-links can further stabilize the absorbed proteins.

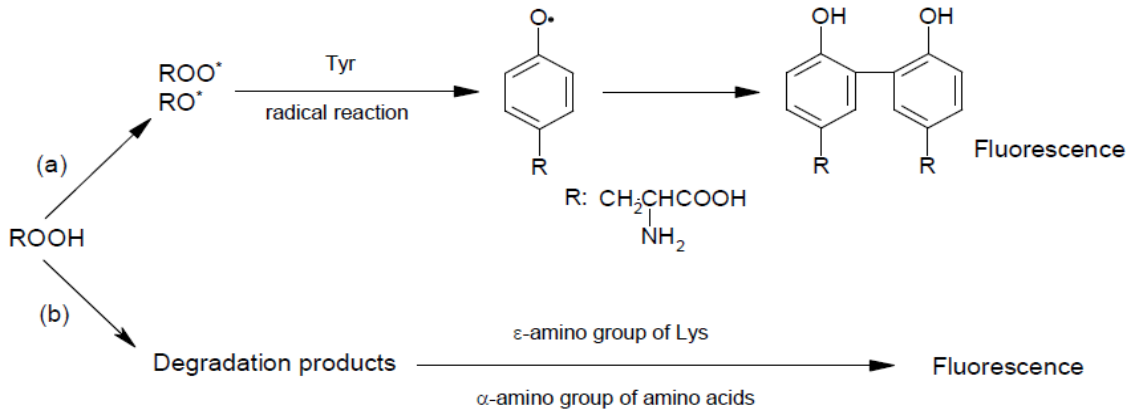

Figure 1.11. Formation of fluorescent compound during protein amino acids interaction with lipid hydroperoxides. (a) The reactive lipid peroxyl (ROO*) or alkoxyl (RO*) radicals react with tyrosine producing fluorescence. The reaction is supposed to occur through radical reaction in which radical abstract hydrogen radical from tyrosine to produce the tyrosine phenoxy radical which in turn condenses to fluorescent dityrosine. (b) The second type of fluorescence is produced by a non-radical reaction in which lipid oxidation degradation products (aldehydes) are reacting directly with amino groups of amino acids leading to fluorescent compounds.
Source: Viljanen, 2005.

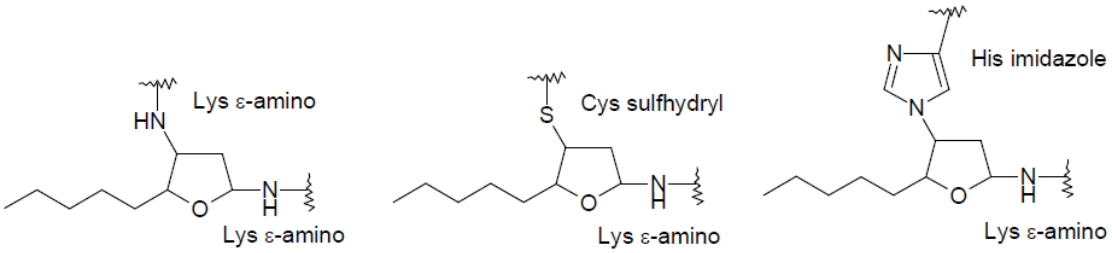

Figure 1.12. Formation of cross-links between different amino acid residues. First the Schiff base is formed between lysine and aldehyde, and then Michael addition of side-chain nucleophiles (cysteine and histidine) will occur.
Source: Viljanen (2005).

1.5.6. Antioxidants

An antioxidant is a substance which, even at low concentrations, delays or inhibits the oxidation of a variety of substrates (Halliwell, 1991; Högberg, 2002). Some antioxidants function as radical scavengers or peroxide decomposers, while others reduce singlet oxygen, remove catalytic metal ions, remove oxygen or inhibit enzymes. The cellular antioxidants in the muscle can be divided into enzymes, water-soluble and fat-soluble low molecular substances.

- *Enzymes and water-soluble antioxidants*

Among the enzymes, the superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione-S-transferase (GST) are all active in skeletal muscle. SOD is a Cu- and Zn-containing enzyme in the cytoplasm and mitochondrial interspace and Mn containing in the mitochondrial matrix (Högberg, 2002). It is known to protect cells against reactive oxygen species and the active centre catalyzes the disproportion of the superoxide anion.

Catalase is the best known enzyme catalyzing the splitting of H2O2 into water and oxygen. Catalase, similarly to SOD, is found in all major organs, but it is more concentrated in the liver and erythrocytes. The catalase activity is very limited in the mitochondria and the endoplasmatic reticulum but is strongly accumulated in the peroxisome and active in the cytosol (Toussaint, Houbion, & Remacle, 1993; Högberg, 2002)

A third enzyme that is also important for antioxidative properties in tissues is the selenium containing GSH-PX which both catalyzes the degradation of H2O2 and lipid peroxides. GPx is found in both the cytosol and the mitochondrial matrix, which allows a more efficient access to H_2O_2. The non selenium containing GST is located in the cytosol and in a small amount in the mitochondrial membranes and endoplasmatic reticulum. This enzyme uses only lipid peroxides as substrate, but the peroxide must be cut out from the membrane by phospholipase A_2 (Högberg, 2002).

Ascorbic acid is a water-soluble, cytosolic antioxidant that exists mostly in the form of ascorbate at physiological pH. This antioxidant is known to be one of the most efficient scavengers of radicals, including lipid radicals (Niki, 1991). Ascorbate can reduce metal ions, react with superoxide radicals, hydroperoxyl radicals, hydroxyl radicals and scavenge singlet oxygen (Halliwell, 1991). Another water-soluble antioxidant is free glutathione (GSH), found in the cytosol and the mitochondria (Halliwell, 1991). It serves as a scavenger for superoxide, hydroxyl radical, and singlet oxygen.

- *Fat-soluble antioxidants*

Vitamin E is divided into the structurally different molecules tocopherols and tocotrienols. Chemically the two forms are different because tocotrienols have an unsaturated phytyl extremity. There are four different forms of both tocopherols and tocotrienols: α, β, χ, and δ. Among the tocopherols α-tocopherol has been recognized as the most important for preventing oxidation changes in meat (Jensen et al., 1998). The γ-tocopherol is also a potent antioxidant (Lynch, 1991; Högberg, 2002), but is not found in large amounts in meat or meat products.

Vitamin E reacts with a large number of molecules that enhance lipid oxidation in muscle in vivo and in meat in vitro (superoxide radicals, hydroperoxyl radicals, hydroxyl radicals, hydrogen peroxide, singlet oxygen and finally, lipid peroxyl and alkoxyl radicals). The basic principle of action of vitamin E is to reduce a lipid alkoxyl and peroxyl radical in the membrane.

Finally, among the lipid-soluble antioxidants found in meat are carotenoids, which can scavenge free radicals in biological systems and protect fatty acids and

membranes against oxidation. The β-carotene is an efficient eliminator of singlet oxygen, hydroxyl radicals, superoxide radicals and peroxyl radicals (Högberg, 2002).

1.6. MEAT NUTRITIONAL PROPERTIES

1.6.1. Meat composition

Meat is an important nutritional source in the diet of most people. It contains a high amount of protein, including essential amino acids, and other beneficial nutrients such as iron and vitamins. Meat is also a major contributor of B vitamins and minerals in the diet (Lawrie, 1998). But the meat also presents some negative effects, such as high content of saturated fatty acids, the intake of which has to be reduced to minimize the adverse effects of cardiovascular disease and the risk of cancer (Parnaud & Corpet, 1997; Ferguson, 2010; McAfee et al. 2010).

- ***Protein***

Meat has a huge amount of protein, which is an important nutrient. Investigations have shown that a diet high in protein can induce weight loss, perhaps because it is more satisfying compared with a carbohydrate-rich diet, so the intake of energy is less (Richelsen, 2006; Aaslyng, 2009). In a world with increasing problems with obesity, this effect could be important.

- ***Muscle lipids***

It is also advised to reduce the amount of fat intake, especially saturated fat (NNF, 2004). Fat from animal sources is very saturated and the authorities in many countries therefore advise the population to reduce the intake of animal fat. This has been taken in by consumers, as many of them nowadays prefer meat without visible fat.

Meat contains variable amounts of fat, depending on the source (chicken, beef, pork, ostrich, fish, etc) and cutting. A large amount of fat can be visible, either between muscles (intermuscular fat) or as the fat layer (subcutaneous fat), and can be removed by the industry before sale or by the consumer before or after cooking. Most meat types,

as ratite meats, are actually very lean and the amount of intramuscular fat should not be reduced too much as this will reduce the eating quality.

According to Aaslyng (2009), not only the amount of fat, but also the fat quality is important with regard to health. The saturated fatty acids, such as lauric (12:0), myristic (14:0) and palmitic fatty acid (16:0), contribute to heart disease by raising plasma low density lipoprotein cholesterol (LDL); whereas linoleic and α-linolenic fatty acids decline it, decreasing the risk of heart disease. Stearic fatty acid (18:0) does not affect plasma cholesterol concentrations, but it may contribute to thrombosis, the final event in chronic heart diseases (CHD) that produces the heart attack. It is therefore included in the ratio of PUFA to saturated fatty acids (P:S) used to assess fat quality in terms of human nutrition and which has an acceptable value of 0.4 or above for the diet as a whole. The P:S ratio for pig muscle is generally above this value but for muscle from cattle and sheep is around 0.1 or less.

The variability in fat content and composition has considerable impact on the product quality of both fresh meat and processed meat products. The effect of feed on fat parameters, e.g. fatty acid composition, antioxidant content and storage stability of meat has been investigated (Wood & Enser, 1997; Nürnberg et al., 1998; Jakobsen, 1999; Lauridsen et al., 1999b; Lauridsen et al., 1999c). The fatty acid composition of tissues in monogastric animals is more sensitive to dietary modification compared with ruminants. Alteration of the fatty acid composition in meat has attracted considerable interest, making meat products more nutritionally favorable (Hartog et al., 1987; Madsen et al., 1992; Enser, 2000). Furthermore, the role of meat as a functional food has been discussed (Jimenez-Colmenero et al., 2001).

- ***Minerals and Vitamins***

Meat plays an important role in supplying iron, zinc, selenium and B vitamins to the diet. The contributions of meat and meat products to total dietary intakes of selected micronutrients are: 14% iron, 30% zinc, 14% vitamin B2, 21% vitamin B6, 22% vitamin B12, 19% vitamin D and 37% of niacin (Mulvihill, 2004). Red meat is rich in iron (e.g., 2.1mg iron per 100 g of fillet steak) and a large proportion of this iron is easily absorbable heme iron. Selenium is a micronutrient also present in meat, which is necessary for some endogenous antioxidative enzymes.

1.6.2. Nutritional characteristics of ratite's meat

The main reference for amino acid and mineral composition of ostrich meat is Sales & Hayes (1996). Ostrich *M. Flexor cruris* and *M. Iliofiburalis* had slightly higher protein, less cholesterol and much lower fat to protein ratio than turkey and beef (Paleari et al., 1998). The fatty acid profile of ostrich meat was generally closer to that of beef than turkey meat (Paleari et al., 1998). Oleic acid (C18:1) is the fatty acid with the highest concentration in ostrich meat, followed by palmitic acid (C16:0) and then linoleic acid (C18:2n-6) (Hoffman & Fisher, 2001; Horbañczuk et al., 1998; Paleari et al., 1998; Sales, Marais, & Kruger, 1996). Ostrich fat might be a source of PUFA or essential fatty acids in human and livestock diets because there is a high PUFA to SFA in breast fat from culled breeding ostrich females (Horbañczuk et al., 2003) and high content of C18:2, C18:3, and C20:4 from breast and back fat depots in 14-month-old ostriches.

According to McMillin & Hoffman (2009), differences in fatty acid content were found in meat from ostriches with 14 months compared with 8 years of age (Hoffman et al., 2001). Within the normal age at slaughtering, the fatty acid profile of ostrich meat varied with bird age (10 to 11 compared with 14 to 15 months) and muscles (*M. Iliofiburalis*, *M. Gastrocnemius*, *M. Iliotibialis*). Muscles from older birds had increased total SFA and MUFA and decreased total PUFA (Girolami et al., 2003).

Differences in individual fatty acids result in different ratios of SFA:MUFA:PUFA as well as n-3:n-6 ratios. In African Black ostriches (*Strutio camelus var. domesticus*), higher percentages of total PUFA were found in the *M. Gastrocnemius* and the *M. Iliofiburalis* (Sales & Hayes, 1996; Sales et al., 1996). The percentage of total SFA, MUFA and PUFA varied between muscles African Black ostrich, with the total SFA (C16:0, C18:0) content being the lowest in the *M. Iliofiburalis* (Sales, 1998). Because the *M. Iliofiburalis* contained the highest total PUFA content, the PUFA:SFA ratio was higher and the MUFA:PUFA ratio was lower than in other muscles. The *M. Gastrocnemius*, *M. Femorotibialis* and *M. Iliotibialis* of ostriches also showed the highest content of n-3-fatty acids (Sales, 1998).

The intramuscular lipids of emu meat showed high levels of linoleic, arachidonic, α-linolenic, and docosahexanoic fatty acids, with a ratio PUFA to SFA of 0.72 (Sales & Horbañczuk, 1998; McMillin & Hoffman, 2009). There were no differences in fatty acids in intramuscular fat among muscles or between Greater and Darwin's rhea species

(Sales et al., 1999). Total SFA content did not differ between species, but MUFA content was lower and PUFA content was higher for Greater rhea than for Darwin's rhea (Sales et al., 1999). The overall fatty acid profile of muscle from *Rhea americana* was similar to ostrich meat and the *M. Gastrocnemius* showed mean content of lipids and cholesterol of 3.87 g and 75 mg per 100 g tissue, respectively (Horbañczuk et al., 2004).

Cholesterol is a structural component of cell membranes and the sub-cellular distribution of cholesterol differs in muscle tissues (Sales et al., 1999); therefore, cholesterol content does not necessarily increase with increased intramuscular fat. Cholesterol content was shown to differ among ostrich muscles in studies by Sales (Sales, 1998), but Horbañczuk et al. (1998) noted no differences between the cholesterol content of the *M. Gastrocnemius* and the *M. Iliofiburalis* (65.63 mg/100 g for both muscles). Cholesterol did not vary with muscle or species in rhea meat (Sales et al., 1999).

Compared with raw meat, cooked ostrich meat *M. Iliofiburalis* to 70 °C decreased the moisture and increased the fat content, with a corresponding increase in cholesterol content from 57 to 72 mg per 100 g (Sales et al., 1996).

Analyzing minerals, heme and non-heme iron contents of rhea meat, Ramos et al. (2009) observed no differences for calcium, phosphorus, magnesium and sodium in *M. Obturatorius medialis* (OM), *M. Iliotibialis lateralis* (IL) and *M. Iliofibularis* (I). They reported that there was more potassium, zinc and copper in IL muscle than in OM and I muscles. For Manganese, OM and IL muscles showed a higher content in comparison with I muscles. For selenium, IL and I muscles showed the highest content compared to OM muscles. For total content of heme and non-heme iron, the IL muscle showed the highest content respect to the other muscles. When compared to other meats, the mineral content of rhea meat showed an elevated level in phosphorus, selenium and total and heme iron content.

1.6.3. Protein aggregation and nutritional implication

The classic criteria for evaluating the quality of a protein source are based on amino acids composition and digestibility of the protein fraction. These basic criteria can only assess the ability of a food to provide globally amino acids. It is now known that the

definition of the quality of dietary proteins needs to integrate new concepts such as: i) the capacity of dietary proteins to release, during the digestion, peptides having a local or systemic biological impact and ii) the rate of digestion which may, in some cases, have a direct influence on whole body assimilation of amino acids.

Protein aggregation results from new interactions between polypeptide chains stemming from oxidation or conformational changes. The formation of carbonyl groups, disulfide bridges or dityrosine, the changes in overall system charge and the increasing hydrophobicity at the protein surface all trigger polymerization and protein aggregation (Davies, 1987; Stadtman, 1990, 1993; Grune, 2004). Furthermore, advanced glycation end-products (products of the Maillard reaction), which form at an increasingly large rate by heating strongly in the presence of sugars (Koschinsky, 1997) also participate in the formation of these protein aggregates. In muscle tissue, myofibrillar proteins and especially myosin tend to establish intermolecular interactions in response to the action of antioxidants (Liu & Xiong, 2000; Kamin-Belski, 1996; Santé-Lhoutellier, 2007) or following heat treatment (Liu & Xiong, 2000; Lefèvre, 2008). The characterization and analysis of the mechanisms underlying the formation of aggregates reveal that the oxidation of myosin leads to the formation of high-molecular-weight structures that conventional polyacrylamide gel-based protein separation techniques are unable to identify. Nevertheless, the size and form of these structures can be studied via techniques based on turbidity and light scattering measurements or microscopy (Fig. 1.13).

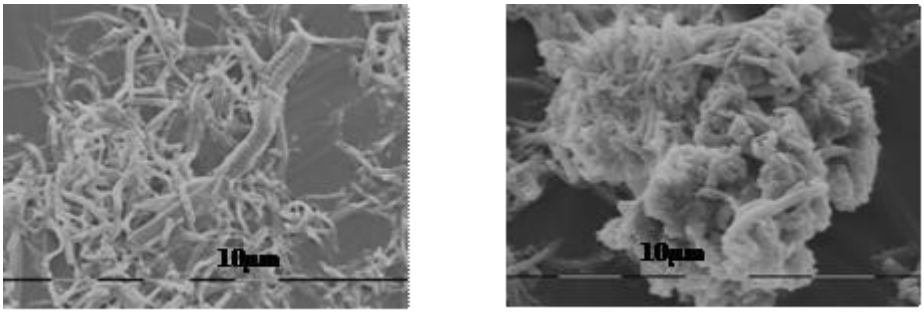

Figure 1.13. Pig myofibrils observed by scanning electronic microscopy before and after heating.

The small bowel digestion of meat proteins has rarely been measured, and there is no data available for assessing the impact of processing treatments on this parameter. However, recent in vitro research suggests that the impact may not be negligible, as it has recently been shown that the degree of protein hydrophobicity influences protein-protein recognition by proteases (Santé-Lhoutellier, 2008b). Thus, whith oxidation of proteins, proteolytic enzymes in the digestive tract can have difficult to recognize protein site of break and, consequently, the digestion of proteins decrease (Fig. 1.14).

Similarly, pepsin, trypsin and chymotrypsin display reduced activity when in the presence of oxidized myosin and in the form of molecular aggregates, and the digestates contain heterogeneous protein fragments of higher molecular weight than the non-oxidized controls (Kamin-Belski, 1996; Liu & Xiong, 2000). Hence, structural and biochemical changes in the proteins could not only impact on digestibility in the small bowel (bioavailability of amino acids vs. colonic fermentation) but also impact on the type of end-products (effect on the release of bioactive peptides) and their rate of digestion.

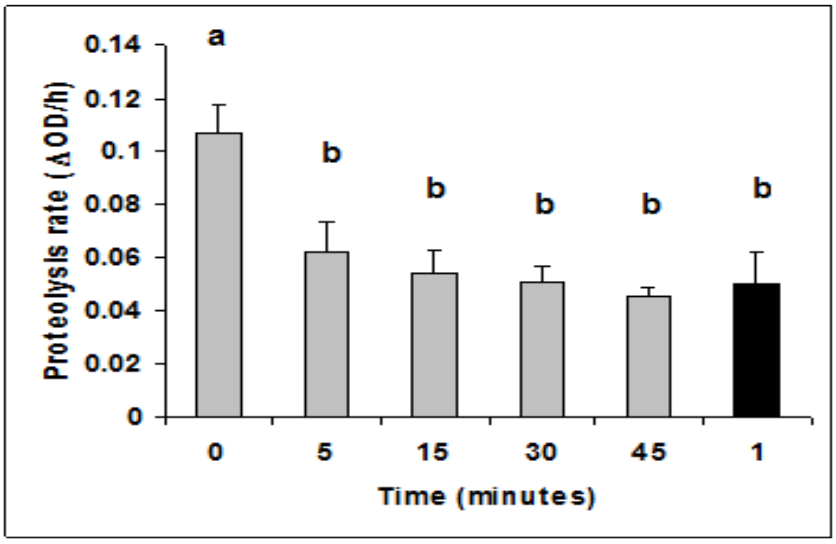

Figure 1.14. Effect of cooking duration (100°C) on myofibrillar proteins proteolysis rate by pepsine. The black bar corresponds to a 270°C cooking for 1 min.
Source : Santé-Lhoutellier et al., 2008.

The absorption and metabolism of ingested modified amino acids from processed foods will vary according to their biochemical modification and the ability of the organs to regenerate the original amino acid. The reaction of lysine with other food components has been examined in detail. Although the first addition product of lysine with reducing sugar or free aldehyde leads to Schiff bases, lysine is still 100% bioavailable in rats. The Maillard reaction continues to Amadori compounds, which are less well absorbed by the gut. The Amadori compounds degrade into small derivatives that can react together or polymerize to produce 'premelanoidins' and 'melanoidins'. Seiquier et al. (2006) demonstrated that a diet rich in Maillard reaction compounds significantly affected protein digestibility.

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Chapter 2

Colour, lipid and protein stability of *Rhea americana* meat during air- and vacuum-packaged storage: Influence of muscle on oxidative processes

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2.1. ABSTRACT

Physicochemical characteristics and oxidative stability during storage were determined in *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF) of *Rhea americana*. Glycolytic potential (GP) and pH decline of muscles were measured within the first 24 h *post mortem*. Colour, lipid and protein stability were determined during storage of meat, i.e. 5 days under air-packaging or 28 days under vacuum-packaging (4 °C). In parallel, the α-tocopherol content, superoxide dismutase and catalase activities, haeminic iron and fatty acids were determined. The ultimate pH was similar in both muscles, but the GP value was significantly higher in IF than in GN muscle. Haeminic iron and alpha-tocopherol content differed between muscles, with 30% more haeminic iron and 134% more alpha-tocopherol in IF than GN muscle. The IF muscle presented higher lipid content and lower polyunsaturated fatty acids/saturated fatty acids ration than GN muscle. With storage under air-packaging, lipid and protein oxidation of rhea muscles increased up to 275% and 30%, respectively. This increase was more rapidly and marked in IF muscle. The IF also showed high level of metmyoglobin accumulation after 3 days of storage (47%) and was rejected by 1 consumer out of 2 in sensorial analysis. Under vacuum-packaging, both muscles showed a high stability of colour and no oxidation of lipids and proteins.

Keywords: Rhea meat; glycolytic potential; fatty acids; colour; oxidation; storage.

2.2. INTRODUCTION

Nowadays, consumers have increasing interest for exotic meat, especially from flightless birds (ostrich, emu and rhea) as a source of proteins for human alimentation. According to the World Ostrich Association (WOA, 2009), current world production of ostrich meat vary between 12,000 and 15,000 metric tons. *Rhea americ*ana is a flightless bird native from South America (Argentina, Bolivia, Brazil, Paraguay and Uruguay) that belongs the ratite family. Among the commercially ratites used for meat production, the rhea market is rather small, but international interest in this new and exotic meat exists and South American farmers consider this as a new commercial possibility (Saadoun & Cabrera, 2008). Most of studies on ratite species have been performed on ostrich and emu for production yield (Morris et al., 1995ab) and meat quality, highlighting the nutritional value of the meat by its low lipid content (Berge, Lepetit, Renerre, & Touraille, 1997; Pegg, Amarowicz, & Code, 2006; Sales, 1996 and 1998; Sales, Navarro, Bellis, Manero, Lizurume, & Martella, 1998). However, information concerning traits of rhea meat is rare and sparse.

Appearance of ratite meat is similar to that of beef: red colour and high level of haeminic iron (Ramos, Cabrera, Del-Puerto, & Saadoun, 2009). Interestingly, it presents high level of unsaturated fatty acids whatever the muscles considered (Sales et al., 1999; Saadoun et al., 2008). Thus, the benefit of this composition requires storage conditions to ensure limited protein oxidation and lipid peroxidation during storage.

Lipid peroxidation producing reactive oxygen species and free radicals is a promoter for myoglobin oxidation, favoring production of rancid odors, off-flavors and surface discoloration of meat (Renerre & Labadie, 1993). Moreover, protein oxidation is also responsible for many biological modifications (Decker, Xiong, Calvert, Crum, & Blanchart, 1993) such as protein fragmentation or aggregation, and for a decrease in protein solubility (linked to sensorial meat quality). The extent of oxidative processes in meat is dependent of the balance between muscle antioxidant molecules such as vitamin E and antioxidant enzymes (catalase, superoxide dismutase and glutathione peroxidase), and lipidic substances sensible to peroxidation (i.e. polyunsaturated fatty acids or PUFA). Recently, Terevintos, Ramos, Castroman, Cabrera, & Saadoun (2009) reported the antioxidant potential of different rhea muscles which seemed to be resistant to in

vitro iron-induced oxidation and presented similar lipid and protein oxidation than other meats, as pig and lamb. For these authors, the oxidative stability of rhea meat is an advantage in comparison to other meats.

The aim of this study was to compare *post mortem* physicochemical attributes and oxidative stability during storage of two muscles of rhea (*M. Gastrocnemius pars interna* and *M. Iliofiburalis*).

2.3. MATERIAL AND METHODS

2.3.1. Animals and samples

The experiment was carried out with rhea *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF), situated in the leg and thigh, respectively. Animals (*Rhea americana*) were reared in a farm in Provins, France, in a semi-intensive production system in which animals were allowed to pasture natural grass in a paddock (cool-season grass like *Lolium perenne*, *Dactylis glomerata*, *Festuca arundinaceae*, *Bromus sterilis*, and *Taraxacum officinale*). In addition to natural grass, a commercial concentrate (based on wheat, oil seed rape, soybean meal and alfalfa pellets) containing 87% of dry matter, 12% of crude protein, 3% of ether extract, 15% of crude fibre, and 10% of ash was offered without restraint to animals. Ten days before the slaughter day, animals were transported from farm to slaughter plant, where they were kept in group and were nourished with the same feed described above. The animals were also allowed to rest before slaughter on a straw-bedded area in a closed barn with sufficient daylight and ensuring minimal disturbance.

Eight 12-month-old animals were manual stunned, i.e. knocked senseless, and slaughtered at the experimental slaughterhouse of the INRA Research Centre of Theix (France). Immediately after slaughter (10 minutes *post mortem*) samples for glycolytic potential and initial pH were taken from both muscles. After that, carcasses were maintained at room temperature (15 °C) for 1 hour and then chilled at 4 °C overnight. Between slaughter and 24 h *post mortem*, additional samples were taken from muscles to determine pH decline and ultimate pH. The GN and IF muscles were removed from carcasses at 24 h *post mortem* and cut into seven 2cm-thick steaks of similar weigh (≈60

g), which corresponded to samples at 1, 3, 5, 7, 14, 21, and 28 days of storage. Samples were packaged under two different conditions: 1) under air permeable film (10000cm3O2 /m2/24h polyvinyl chloride film) during 5 d. at 4 °C; 2) vacuum-packaged during 28 d. at 4 °C.

Supplementary samples were also taken from both muscles immediately after deboning, frozen into liquid nitrogen and stored at -80 °C for chemical composition and biochemical analyses of muscles. Colour parameters, lipid oxidation, protein oxidation, and drip loss were determined at 1, 3 and 5 d. in samples stored under air-packaging and at 7, 14, 21 and 28 d. of storage in vacuum-packaged samples.

2.3.2. Muscle pH and temperature

Muscle homogenate pH was measured 10 min, 1 h 30, 3 h, 5 h and 24 h after slaughter. Samples (2 g) were taken in the medial portion of muscles and homogenized with 18 mL of 5 mM sodium iodoacetate buffer. Homogenate pH was measured using a pH meter (Hanna instruments, Woonsocket, USA) equipped with a combined electrode. Muscle temperature was recorded at the same time than for homogenate pH.

2.3.3. Muscle glycogen, lactate and glycolytic potential

Glycogen, glucose, glucose-6-phosphate and lactate of muscle homogenate were measured at 10 min, 24 h, and 3 days after slaughter were measured by enzymatic procedures according to Dalrymple & Hamm (1973) and Bergmeyer (1974) with slight modifications. Muscle tissue (1 g) was homogenized with 10 mL of 0.5 M percloric acid. Aliquots of homogenate (0.5 mL) were taken for enzymatic determination of glycogen, glucose and glucose-6-phosphate after glycogen hydrolysis with amyloglycosidase. Lactic acid was determined in the supernatant resulting from the centrifugation at 20 min at 4000g of homogenate. Results were expressed in micromoles per gram of fresh tissue.

The glycolytic potential (GP, i.e. the amount of compounds transformable into lactic acid) was measured at 10 min, 24 h and 72 h *post mortem* and expressed as

micromoles of lactate equivalent per gram of fresh tissue. Calculation was done according to the following formula (Monin & Sellier, 1985):

GP = 2 x ([glycogen] + [glucose 6-phosphate] + [glucose]) + [lactic acid]

2.3.4. Fatty acid analysis

Muscle tissues were mixed in N2 liquid to produce a homogenous and fine powder stored at -20 °C until lipid and fatty acids (FA) analysis. Total lipids of GN and IF muscles were extracted by mixing 5 g of meat powder with chloroform/methanol (2:1, v/v) according to the method of Folch, Lees, & Stanley (1957) and determined gravimetrically. Fatty acids were extracted from muscle total lipids and transmethylated at room temperature first with sodium methylate (1 M) and second with boron trifluoride in methanol (14%, v/v). The qualitative and quantitative analysis of fatty acids was achieved by gas-liquid chromatography (GLC) using the Perichrom 2000 chromatograph (Perichrom, Saulx-les-Chartreux, France) fitted with the CP-Sil 88 glass capillary column (length: 100 m, i.d.: 0.25 mm) with H2 as the carrier gas. The chromatographic conditions were as follows: the oven temperature was set at 70 °C for 30 s, then ramped from 70 to 175 °C at 20 °C/min, held at 175 °C for 25 min, ramped from 175 to 215 °C at 10 °C/min, and finally held at 215 °C for 41 min; injector and detector temperatures were 235 and 250 °C, respectively. Hydrogen was the carrier gas (H_2 flow: 1.1 mL/min) in conditions of split injection (1:50). FAs were quantified by using C19:0 as the internal standard (Supelco, Bellefonte, PA). Response coefficient of each individual FA was calculated by using the quantitative mix C4-C24 FAME (Supelco, Bellefonte, PA).

2.3.5. Alpha-tocopherol (vitamin E)

Tissue concentration of α-tocopherol was measured by HPLC as the method of Hatam & Kayden (1979) adapted for bovine by Scislowski, Bauchart, Gruffat, Laplaud, & Durand (2005). Before its extraction, 400 µL of tocopherol acetate (internal standard) was added to tissue samples to determine the extraction yield. Samples were then treated with KOH in ethanol 11% and heated at 80° C into a water bath for 20 min. Then, α-tocopherol was extracted twice with hexane. Hexane was eliminated by evaporation

and α-tocopherol + tocopherol acetate solubilized with 160 µL tetrahydrofuran and 240 µL methanol/dichloromethane solution (65/35, v/v) and separated by the HPLC system (Kontron Analysis Division, Zurich, Switzerland) equipped with nucleosil 5 µ C18 column (250mm×4.6 mm) (Interchim, Montlucon, France) with methanol as the mobile phase (flow rate of 1.5 mL/min). Alpha-tocopherol and tocopherol acetate were determined by UV spectrophotometry at 292 nm using HPLC detector model 430 with the "Kroma System 2000" software (Kontron Analysis Division, Zurich, Switzerland).

2.3.6. Antioxidant enzyme activities

Antioxidant enzyme activities were measured in frozen meat samples (-80 °C). Muscle samples (1 g) were homogenized with 10 ml of 50 mM phosphate buffer (pH 7.0) with a Polytron and centrifuged at 4000g for 15 min at 4 °C. Protein concentration was determined by the biuret method (Gornall, Bardawill, & David, 1949). Total superoxide dismutase activity SOD (Cu–Zn SOD and Mn SOD) was measured according to the procedures of Marklund & Marklund (1974) using inhibition of pyrogallol autoxidation in a basic medium. One unit (U.) was taken as the activity that inhibits the pyrogallol autoxidation by 50%. Catalase activity was measured by the rate of disappearance of H2O2 at 240 nm following the method of Aebi (1974). Catalase activity was expressed as nmol of decomposed H2O2/min/mg protein.

2.3.7. Drip loss

The drip loss was determined post-cooling by gravimetry and the results were expressed as average proportion (Honikel, 1998).

2.3.8. Colour

2.3.8.1. Spectrophotometry

Muscle colour was assessed in fresh meat. Prior to instrumental colour evaluation of vacuum-packaged meat, samples were removed from their packages and held under air for 2 h at 4 °C. Visible reflectance spectra (from 360 to 760 nm) were determined with an Uvikon 933 (Kontron) spectrophotometer equipped with an integrating sphere.

Reflectance spectra were collected from a 2° viewing angle and with illuminant D65 (Daylight) lighting conditions. Colour coordinates were expressed as lightness (L*), redness (a*) and yellowness (b*). Oxygenation index (ΔR) was determined by reflectance difference between 630 nm (maximum of the oxidized myoglobin) and 580 nm (maximum of the oxygenated myoglobin) (Renerre, 2000). The metmyoglobin percentage (% MetMb) at the meat surface was determined by the method of Krzywicki (1979).

2.3.8.2. Myoglobin content

Haeminic iron was determined on 5 g of each muscle sample using the method of Hornsey (1956). Values were expressed as μg of iron per g of meat (ppm).

2.3.8.3. Sensory

The sensory colour of muscles was accessed in fresh meat. Samples under air-packaging at 1, 3 and 5 d. of storage were evaluated by sensory panel according to Goñi, Indurain, Hernández, & Beriain (2007), with slight modifications. A total of 10 no-trained panelists used 5-point scales to evaluate the meat redness (5 = bright purplish red to 1 = brown), the meat lightness (5 = light to 1 = dark), the meat aspect (5 = pleasant to 1 = unpleasant) and their purchase intention (yes or not).

2.3.9. Lipid oxidation

Lipid oxidation was measured by the thiobarbituric acid-reactive substances (TBA-RS) method according to Lynch & Frei (1993) modified by Mercier, Gatellier, Viau, Remignon, & Renerre (1998). Muscle samples (1 g) were homogenized with 10 ml KCl 0.15 M + BHT 0.1 mM with a Polytron (1 min, medium speed). Homogenates (0.5 mL) were incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH (0.25 mL) and 2.8% (w/v) trichloroacetic acid (0.25 mL) in a boiling water bath for 10 min. After cooling at room temperature for 20 min, the pink chromogen was extracted with n-butanol (2 mL) and its absorbance measured at 535 nm against a blank of n-butanol.

TBA-RS concentrations were calculated using 1,1,3,3 tetrathoxypropane (0-0.8 μM) as standard. Results were expressed as mg of MDA per kg of meat (TBA units).

2.3.10. Carbonyl content

Muscle samples (1 g) were homogenized in 10 ml KCl 0.15 M + BHT 0.1 mM with a Polytron (1 min, medium speed). Proteins were treated with DNPH (2,4 dinitrophenylhydrazine) using the method of Oliver, Alin, Moerman, Goldstein, & Stadtman (1987) modified by Mercier et al. (1998). Results were expressed as nanomoles of DNPH fixed per milligram of protein.

2.3.11. Statistical analysis

Analysis of variance (ANOVA) was performed using the General Linear Model (GLM) procedure of SAS system. The linear model included fixed effects of time and muscle. When significant effect of muscle and time were encountered, least squares means were compared using LSMEANS with PDIFF option and TUKEY adjustment. The relationships between the different parameters were assessed by calculating Pearson correlation coefficients.

2.4. RESULTS AND DISCUSSION

2.4.1. Muscle pH and temperature

The pH and temperature evolution of rhea *M. Gastrocnemius pars interna* and *M. Iliofiburalis* is presented in Fig. 2.1. The decline of pH and temperature did not differ according to the muscles, with an ultimate pH average comprised between 5.4 and 5.6, and final temperature of 4 °C at 24 h *post mortem*. Sales et al. (1998) found an ultimate pH at 24 h *post mortem* higher in the same rhea muscles (5.99 and 5.76 for GN and IF muscles, respectively) than the ultimate pH found in this study. According to the results reported by Paleari et al. (1998) on ostrich *M. Iliofiburalis*, ratite meat acidification is closer to beef meat (loin, pH = 5.8) than to poultry (thigh, pH = 6.3).

Other studies have also reported an intermediate to high ultimate pH in emu (between 5.6 and 6.4; Berge et al. 1997) and ostrich meat (pH average of 6.1; Seydim,

Acton, Hall, & Dawson, 2006). This difference in ultimate pH can be explained by the pre-slaughter conditions. In present experimental conditions, ante mortem stress was limited because the rheas were not transported before slaughter. Such pre-slaughter conditions minimized the ante mortem glycogen depletion and consequently allowed a complete *post mortem* acidification. However, the rate of pH and temperature decline of both rhea muscles was rapid immediately after dead: 10 minutes after slaughter the average pH was 6.2 and muscle temperature was about 41.5 °C. Similar rate was reported by Sales et al. (1998) for rhea muscles and by Sales & Mellett (1996) in ostrich muscles. For Leche, Busso, Hansen, Navarro, Marin, & Martella (2009) ratites exhibited higher hormonal response to stress that it is usually found in birds.

2.4.2. Glycogen, lactate and glycolytic potential

Glycogen, lactate and glycolytic potential evolution are presented in Table 2.1. After slaughter, glycogen amount decreased in both muscles. Initial and final glycogen concentration differed between muscles ($p < 0.0001$) with highest values found in IF muscle. Indeed, 10 minutes after slaughter, glycogen concentration in GN and IF muscles were 25 µmol/g and 59 µmol/g, respectively. Lactate accumulated rapidly in both muscles, with an average of 45 µmol/g 10 minutes *post mortem*, demonstrating a high rate of glycolysis activity. The muscle residual glycogen remained stable between 1 d. and 3 d. of storage, with a high content in IF than in GN muscle. The glycogenolysis process in rhea muscles stopped before complete depletion of muscular glycogen. Despite the difference in glycogen content at slaughter, there was no difference in ultimate pH between rhea muscles. In turkey muscle, Fernandez et al. (2001) also observed a stop of pH decline while the glycogen in turkey muscles was still present. It is well known that the relationship between glycogen content at slaughter and ultimate pH is not linear (Bendall, 1973). At a given pH value, depending on species and muscles, *post mortem* glycolysis stops even in presence of residual glycogen. The mechanisms explaining this phenomenon have been discussed by Scopes (1971). According to this author, the disappearance of adenosine monophosphate (AMP) in *post mortem*, a cofactor of glycogenolytic and glycolytic enzymes, would explain the cessation of pH decline in the presence of glycogen.

The glycolytic potential was higher in IF muscle (≈164 eq. lactate/g tissue) than in GN muscle (≈93 eq. lactate/g tissue). No available data on glycolytic potential of ratite muscle was found in literature. However, in *M. Longissimus dorsi* of Large White pigs, Monin, Mejenes-Quijano, Talmant, & Sellier (1987) found a GP value of 170 μmol/g meat. In chicken, LeBihan-Duval et al. (2008) reported a GP value equal to 108 μmol eq. lactate/g tissue in breast muscle (*M. Pectoralis major*), and in bovine muscle Wulf, Emnett, Leheska, & Moeller (2002) reported a GP value of 122 μmol eq. lactate/g muscle.

2.4.3. Muscle lipid and alpha-tocopherol (vitamin E)

Total lipid content and centesimal composition of their fatty acids (FA) in rhea *M. Gastrocnemius pars interna* and *M. Iliofiburalis* are shown in Table 2.2. Total lipids and cholesterol contents were 1.9 and 1.5 times higher in IF muscle than in GN muscle ($p < 0.05$), in agreement with previous data reported for Blue Neck (*Struthio camelus australis*) ostrich muscles (Girolami, Marsico, D'Andrea, Braghieri, Napolitano, & Cifuni, 2003). On the other hand, Horbańckuk, Sales, Celeda, Kanecka, Zieba, & Kawka (1998) did not find any differences in total lipid content between *M. Gastrocnemius* and *M. Iliofiburalis* of Red Neck (*Struthio camelus massaicua*) and Blue Neck (*Struthio camelus australis*) ostriches.

In this study, linoleic acid (C18:2n-6) was quantitatively the main fatty acid followed by oleic acid (C18:1) and then palmitic (C16:0). Similar information was reported by Romanelli, Trabuco, Scriboni, Visentainer, & Souza (2008) in GN muscle of rhea. This differed from data reported for ostrich meat, in which C18:1 was the fatty acid with highest concentration followed by C16:0 and C18:2n-6 (Horbańczuk et al., 1998; Paleari et al., 1998; Sales, 1994; Sales, 1998).

The percentage of saturated fatty acids (SFA) was different between the two muscles: 29.88% for GN muscle and 34.44% for IF muscle. On the contrary, the percentage of polyunsaturated fatty acids (PUFA) was higher in GN than in IF muscle (45.15% vs. 39.09%). Saturated fatty acid C16:0 was 1.3 times higher in IF muscle than in GN muscle, whereas 18:0 did not differ between the two muscles. Therefore, the C16:0/C18:0 ratio was 1.4 times lower in GN than in IF muscle, indicating a better health

value of saturated FA in GN than in IF muscle. The C16:0 is considered more detrimental for human health due to their atherogenic properties compared to C18:0 that is more neutral for this disease (Spady, 1993).

Concerning PUFA, analysis of the composition of n-6 and n-3 PUFA showed that the amount of n-3 PUFA was 1.3 times higher in GN ($p < 0.001$) than in IF muscle, especially C20:5 n-3 (x 1.5), C22:5 n-3 (x 1.9) and C22:6 n-3 (x 4.8) ($p < 0.001$). Thereby, the n-6 PUFA/n-3 PUFA ratio was 1.2 times lower in GN muscle than IF muscle, indicating that PUFA composition in GN muscle was more positive to consumer's health than PUFA composition in IF muscle. However, values for this ratio (6.8 and 8.1) were, in both muscles of rhea, higher than the value recommended for humans (5.0).

The values for the ratio PUFA/SFA were particularly high in rhea muscles (1.15-1.52). In GN muscle it was 1.3 times higher than in IF muscle. The values were higher than 0.4, the recommended value for human health (Wood et al., 2004) and they were also higher than the PUFA/SFA values reported in beef (0.15-0.31; Bauchart et al., 2005), and chicken broiler breast meat (0.7; Soares et al. 2009).

Such differences in FA composition of GN muscle compared to that of IF muscle can be associated to the difference observed in their lipid content. The higher lipid content in IF muscle compared with GN muscle was probably due (as in muscles of other animal species) to the result of a higher deposition of triglycerides (TG), which are known to be rich in saturated FA and relatively poor in PUFA (Scollan et al., 2005). Such increase in TG would lead to the alteration of the health value of fatty acids in IF muscle compared to that in GN muscle. Meats containing greater contents of highly unsaturated lipids are likely to be more predisposed to lipid oxidation than those containing more saturated fatty acids and therefore may have an increased requirement for anti-oxidants. Thus, the α-tocopherol concentrations in fresh meat of rhea are presented in Table 2.2. The vitamin E content was 2-folds greater in IF muscle than in GN muscle ($p = 0.0002$). However, the α-tocopherol concentration values were very low (< 1.0 μg/g tissue). In beef, the concentrations of vitamin E in pasture-fed and grain-fed animals (not supplemented in α-tocopherol), were 4.5 and 1.8 μg/g muscle, respectively (Yang, Lanari, Brewster, & Tume, 2002).

2.4.4. Antioxidant enzyme activities

Table 2 shows superoxide dismutase (SOD) and catalase activities. The SOD activity did not differ between muscles. This enzyme catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide. As such, SOD is an important antioxidant defense in nearly all cells exposed to oxygen. In bovine muscles, Renerre, Dumont, & Gatellier (1996) found the highest SOD activity in M. Diaphragma (> 3.0 units at 1 d. *post mortem*) when compared to *M. Longissimus lumborum* and *M. Tensor fasciae latae*. In rhea muscles immediately after slaughter, Terevinto et al. (2009) found significantly lower SOD activity in *M. Iliofiburalis* (≈0.8 units) in comparison with *M. Iliotibialis lateralis* (≈1.0 units), but not to *M. Obturatorius medialis* (≈0.9 units). Their values were lower than the values found in this study.

The catalase activity differed between muscles ($p < 0.01$) (Table 2). Catalase catalyses the dismutation of hydrogen peroxide in water and oxygen. Meat from the IF muscle had a higher catalase activity than meat from GN muscle. The result for catalase activity in rhea IF muscle found in this study was similar to those reported by Terevinto et al. (2009). These authors reported ≈5000 nmol H2O2/min/mg protein in IF muscle and they did not find differences in catalase activity between IF muscle, *Iliotibialis lateralis* and *Obturatorius medialis* muscles. Jenkis & Tengi (1981) found that slow oxidative muscles exhibited a significantly greater catalase activity than fast oxidative muscles. In beef (Renerre et al., 1996) and in turkey (Renerre, Poncet, Mercier, Gatellier, & Metro, 1999) it was also observed that more oxidative muscles had higher catalase activity than less oxidative muscles. According to these authors, this could indicate that oxidative muscles have a natural protection against the deleterious effects of oxy-radicals (particularly the superoxide anion).

2.4.5. Muscle drip loss

During storage under air, drip loss increased for both muscles, but it was significantly higher in IF than in GN muscle (from 1 to 1.8% in GN muscle and from 1.8 to 3.5% in IF muscle after 5 d. storage, $p < 0.0001$). The ability of fresh meat to hold on to its own water (water-holding capacity) is an important quality aspect, since it affects consumer acceptance and final weight of the product. Hertog-Meinschke, Smulders, &

Logtestijin (1998) reported 1.75% of drip loss after 3 days under air-packaged storage in bovine *M. Semimembranosus*. Several factors can influence the amount of drip. Besides genetics, other aspects, including animal and carcass handling, temperature management *post mortem*, nutrition, and processing, can also play an important role on drip loss (Jennen et al., 2007). Under vacuum-packaged storage, initial drip loss did not differ between muscles, but it doubled after 21 d. of storage (4% for GN muscle and 6% for IF muscle).

2.4.6. Colour parameters

Colour coordinates (L*, a*, b*, ΔR) and iron contents are shown in Table 2.3 for air- packaged meat and in Table 2.4 for vacuum-packaged meat.

Lightness L:* Under air, lightness did not change in GN muscle, while in IF muscle a significant decrease of L* values was noted after 5 d. of storage. In this study, initial L* was similar to L* values reported by Seydim et al. (2006) in ground ostrich meat and by Paleari et al. (1998) in ostrich meat, even if in the last study the authors did not distinct IF muscle from *M. Flexo cruris*. L* values in rhea meat were also similar to L* values reported in beef *M. Longissimus dorsi* (Gatellier, Mercier, Juin, & Renerre, 2005). The fresh ostrich meat (IF muscle) had similar L* values than beef and was darker than turkey, while the cooked ostrich meat was darker than turkey and beef (Paleari et al., 1998). For vacuum-packaged meat, lightness L* remained stable during storage for GN muscle, while a slight decrease of lightness was observed in IF muscle during storage between 21 and 28 days. Lightness values for vacuum-packaged rhea meat were slightly lower than those reported by Otremba et al. (1999) in vacuum-packaged ostrich meat previously frozen (*M. Gastrocnemius*) and Seydim et al. (2006) in vacuum-packaged ground ostrich meat (*M. Gastrocnemius* and *M. fiburalis longus*), but grinding and freezing may affect the integrity of muscle cell and consequently increase L* values.

Redness a:* Redness is the most important colour parameter to evaluate meat oxidation. Prolonged storage under air-packaging induces the transformation of oxymyoglobin (bright redcolour) into brown metmyoglobin. This change decreases the redness a* and makes the meat unacceptable for the consumers (Renerre, 2000). In this study, under air-packaging, redness of samples differed according to the muscle and

storage time. In GN muscle stored under air-packaging, redness a* remained stable during storage, while in IF muscle regular decrease of a* was observed. The decrease in this colour parameter indicates that meat become less red and vice-versa. Comparing the results obtained in the present study to those reported by Paleari et al. (1998), ostrich, turkey and bovine meats seem to be redder than rhea meat. The instrumental a* values reported by these authors were higher than a* values found in this study. According to Seydim et al. (2006), a decrease in redness is due to myoglobin oxidation, especially when meat pH is above 6. At high pH values, mitochondrial enzyme systems (cytochrome, succinate and pyruvate-malate oxidase) do not shut down and have the ability to utilize available oxygen (Lawrie, 1998). Bendall & Taylor (1972) reported that oxygen consumption rate of high pH muscle is higher than normal pH muscle. Bembers & Satterlee (1975) also noted that the rate of the conversion of oxymyoglobin into metmyoglobin was 1.5-2.0 times faster in high pH systems than in muscles having more normal pH.

Under vacuum-packaging, redness did not differ between muscles neither during storage (Table 4). Both muscles presented high stability during four weeks of storage. On this study, the redness values a* were in general higher in vacuum-packaged meat than in air-packaged meat, whatever the muscle considered.

Yellowness b:* We observed that yellowness did not differ between muscles neither during storage time for air-packaged rhea meat. Pollok et al. (1997) found b* values of 1.90 and 3.51 for ostrich *M. Gastrocnemius pars externa* and *M. Iliofiburalis*, respectively. Paleari et al. (1998) reported an average of 6.6 in whole thigh muscles and Seydim et al. (2006) showed b* equal to 6.3 in air-packaged meat of ostrich at 0 day. Under vacuum, ANOVA revealed an effect of muscle on yellowness b*. The b* value (7 d.) found in GN muscle was significantly different ($p < 0.0001$) than final b* values found in IF muscle (21 d. and 28 d. of storage).

ΔR or R630-R580: A decrease of R630-R580 reflects meat discoloration and metmyoglobin formation. Initial R630-R580 values were similar in both GN and IF muscles. Under air-packaged we observed a decrease in R630-R580 of rhea muscles, but in IF muscle the decrease was more important than in GN muscle. Indeed, R630-R580 values reached 2.4 after 5 d. of storage, which corresponded to 85% of decrease. For emu meat, Berge et al. (1997) found similar results, with a decrease of 50% and 65% in

R630-R580 after 3 and 8 d. of display, respectively. In beef *M. Longissimus dorsi*, Gatellier et al. (2005) never reached such myoglobin oxidation level when they applied 14 d. of storage under vacuum-packaging followed by 6 d. of air-packaged storage.

Overall, under vacuum-packaging the GN and the IF muscle showed similar R630-R580 values during all period of storage, highlighting the absence of surface discoloration and myoglobin oxidation in rhea meat. Only a small decrease in IF muscle was noted between 7 and 28 d. of storage.

Metmyoglobin percentage: The evolution of surface metmyoglobin in air-packaged samples is presented in Fig. 2.2. The initial %MetMb did not differ between muscles, but during storage it was noticed a higher increase in this parameter in IF than in GN muscle. Similarly, Gatellier, Hamelin, Durand, & Renerre (2001) reported an increase of 45% of MetMb accumulated on air-packaged beef (*M. Longissimus lomborum*) during 9 days of storage under air-packaging.

Haeminic iron: Haeminic pigment can be categorized as a pro-oxidant substance because its oxidation has been described to be a major catalyst of free radical formation such as superoxide radical (Kanner, Harel, & Granit, 1992) and peroxyl radical (Kanner & Harel, 1985). The rhea IF and GN muscles differed in their amount of haeminic iron, with 30% more haeminic iron in IF muscle (Table 3). Recently, Ramos et al. (2009) reported lower amount of iron in IF muscle of rhea than we observed. The results reported in this study for muscle IF are similar to those reported by Patak & Baldwin (1993) and Berge et al. (1997) in emu meat and Sales (1996) in ostrich meat, with concentration of haeminic iron between 26 and 29 μg/g. The rhea meat showed a high level of haeminic iron in comparison to chicken, beef, lamb and pork (Lombardi-Boccia, Martínez-Domínguez, Aguzzi, & Rincón-León, 2002).

Sensory colour: The results of visual sensory analysis of air-packaged rhea meat are shown in Table 2.3. As reported above with instrumental colour measurements, GN muscle aspect remained stable during storage (sensory lightness, redness and aspect) while in IF muscle these parameters decreased during storage time. After 3 d. of storage, more than 60% of the jury rejected the meat from IF muscle. At 5 d., while for GN muscle the purchase intention was above 87%, it was less than 5% for IF. The meat was judged too dark and brown. The correlation coefficients between R630-R580 and sensorial lightness and between R630-R580 and redness were highly significant ($r = 0.91$, $p <$

0.0001 and r = 0.91, p < 0.0001, respectively). At 5 d. of storage, the Pearson correlation coefficient between redness a* and "intention of purchase" attribute was also highly significant (r = 0.92; p < 0.0001). These results confirmed that colour influences meat purchasing decisions and consumers use discoloration as a negative indicator of freshness and wholesomeness (Mancini & Hunt, 2005). Consumers reject meat with high contents of MetMb (Van den Oord & Wesdorp, 1971; Hood & Riordan, 1973; MacDougall, 1982; Renerre & Mazuel, 1985). The strategy for maximizing acceptable colour (and colour shelf life) involves delaying pigment oxidation and/or enhancing reduction of oxidized pigment. Considerable evidence exists that anoxic packaging systems can extend the storage-life of red meats (Jeremiah & Gibson, 2001), but this also can increase the drip during storage, as we noticed.

2.4.7. Oxidative parameters

The changes in TBA-RS values and carbonyl contents of rhea GN and IF muscles during air-packaged storage are shown in Table 2.3, and under vacuum-packaged storage in Table 2.4.

Lipid oxidation: The TBA-RS values at 1 d. *post mortem* did not differ between muscles. In GN muscle, TBA-RS remained stable during storage time, while in IF muscle we observed a remarkable increase of TBA-RS (432% higher) during 5 d. of storage. Under similar storage conditions, Gatellier et al. (2001 and 2005) observed only an increase of 50-75% on TBA-RS values in beef (final values near 2.2 mg MDA/kg tissue). The results reported in this study for GN muscle are similar to those reported by Seydim et al. (2006), who found TBA-RS values for ground ostrich meat under air-packaging lower than 4.0 mg MDA/kg tissue at 5 d. of storage. Recently, Terevinto et al. (2009) reported the mean of 0.84 mg MDA/kg tissue in rhea muscles at 0 d. *post mortem*, and the IF was the muscle that presented the lowest TBA-RS value (≈0.6 mg MDA/kg tissue) in comparison to other rhea muscles. In this study, TBA-RS values (at 7 days) in vacuum-packaged meat were lower in GN muscle than in IF muscle. During the storage under vacuum-packaging, no evolution of this parameter was observed. The TBA-RS values remained under 2 mg MDA/kg tissue whatever the muscle or the time considered. Similarly, Fernandez-Lopez, Sayas-Barberá, Muñoz, Sendra, Navarro, & Perez-Alvarez

(2008) have not observed differences in TBA-RS values during vacuum-packaging storage of ostrich steaks.

Protein oxidation: Under air, carbonyl contents at 1 d. *post mortem* did not differ between the two muscles of rhea. In GN muscle carbonyl content remained stable during air-packaged storage, while in IF muscle we observed an accentuated increase in carbonyl content after 5 d. (about 60%). The results at 1 d. *post mortem* were similar to the results reported by Terevinto et al. (2009) for rhea *M. Obturatorius medialis* at 0 day. For rhea *M. Iliofiburalis* (IF) these authors reported 0.79 nmol/mg protein. In the muscle Sartorius of turkey, Mercier et al. (1998) found high carbonyl content (about 3.15 nmol/mg proteins) after 9 d. of storage under air-packaging. For beef, the values reported by Insani, Eyherabide, Grigioni, Sancho, Pensel, & Dascalzo (2008) were lower than the values reported in this study. During 9 d. of storage these authors found a slight increase in carbonyl content in beef, but the values were always lower than 2 nmol/mg of protein.

Similarly to lipid oxidation, under vacuum-packaged storage the carbonyl content did not change with storage time, evidencing the absence of protein oxidation.

2.4.8. Oxidative processes in rhea meat

The results reported in this study demonstrated clearly that oxidative processes can be avoided by removing oxygen during storage. Muscle composition (lipid profile and antioxidant components) can partly explain the differences between the two studied muscles. Nevertheless, our results highlight the possible effect of residual glycogen. In IF muscle, the amount of residual glycogen was 4 times higher than in GN muscle. We observed positive correlations between residual glycogen concentration and myoglobin oxidation ($r = 0.86$; $p < 0.0001$), TBA-RS values ($r = 0.81$; $p < 0.001$), or carbonyls content ($r = 0.72$; $p < 0.01$) at 5 d. of storage. Glycogen would not directly promote oxidation, but could act as a precursor of glucose, which is implicated in advanced glycation end products (AGEs). Further research are needed to study the mechanisms by which glycogen and its "by products" could enhance oxidative processes during air-packaged storage of meat.

2.5. CONCLUSION

Rhea *M. Gastrocnemius pars interna* and *M. Iliofiburalis* exhibited different stability under air-packaged storage. In particular, IF muscle presented higher oxidation processes and instability than GN muscle, altering its quality attributes. It can be partly explained by the fatty acid composition and the higher myoglobin content in IF than in GN muscle. Besides, the residual glycogen of rhea muscles also seems to be involved in the occurrence of the oxidative process, but it remains unclear and need to be well investigated. Under vacuum-packaging, both muscles did not present evidence of oxidation.

In terms of oxidative processes, the results obtained in this study indicate that to ensure the quality of the rhea meat and to keep the uniformity of the product offered to consumers, Rhea meat industry should prioritize the adoption of vacuum-packaged storage instead of air-packaged storage. The exposure of rhea meat to the air was extremely degenerative and prejudicial to the IF muscle quality, while the employment of vacuum-packaging was appropriate and could efficiently control the oxidation of myoglobin, lipids and proteins. This data is interesting because it provides important information to the industry, and enables it to better choose packaging and to better offer its product in the market. However, more complete and comprehensive studies on the characterization of other muscles of rhea, the microbiological quality of rhea meat during storage and the use of modified atmosphere packaging are very necessary. The knowledge about characteristics and peculiarities of rhea meat will allow the industry development, the marketing of meat and, consequently, the acceptance and trust of the consumer.

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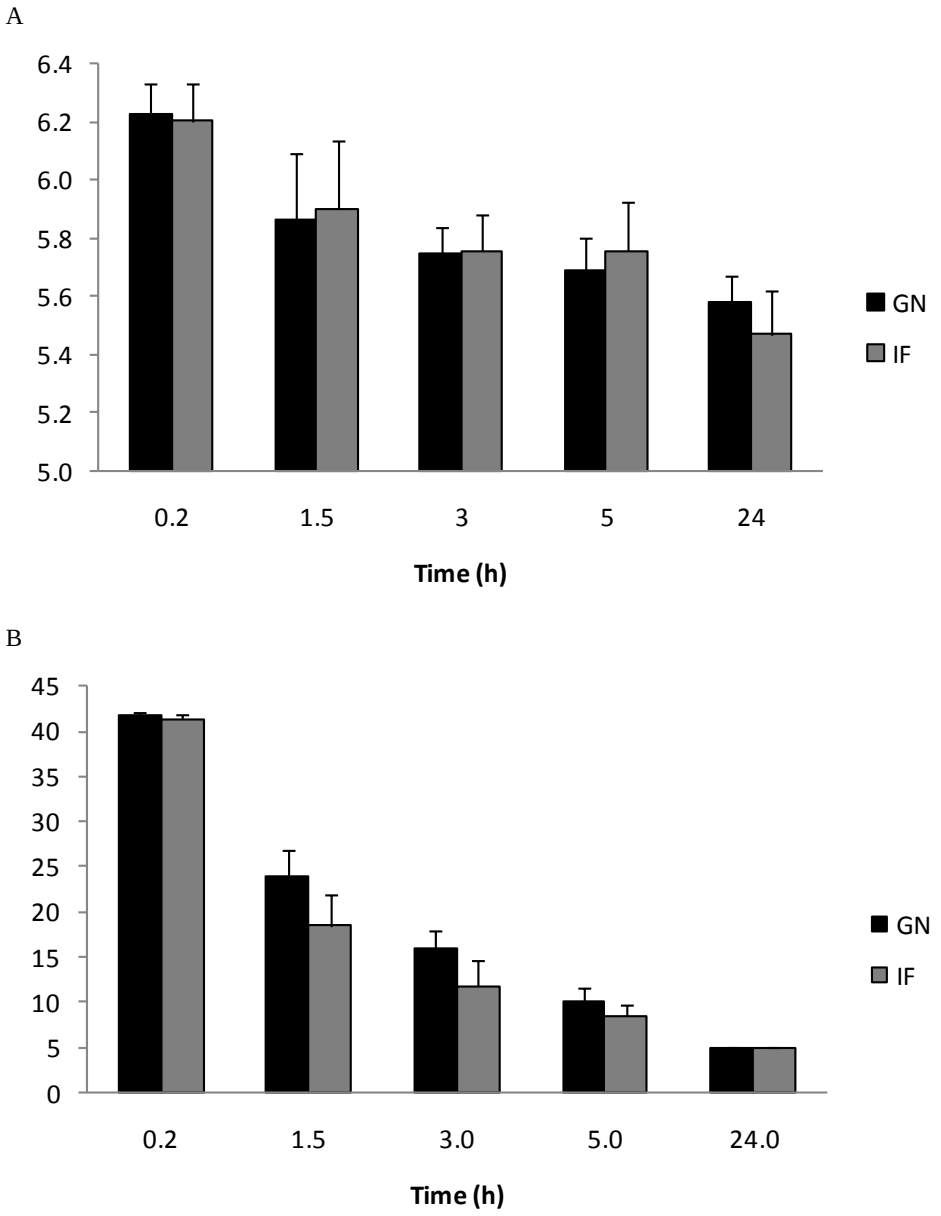

Figure 2.1. *Post mortem* pH (A) and temperature (B) decline in *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF) of *rhea americana*, least squares means and standard deviation.

Table 2.1

Means (±SD) for metabolic parameters in two muscles of *rhea americana*.

	Metabolic parameters		
	Glycogen (µmol/g tissue)	**Lactate (µmol/g tissue)**	**GP* (eq. lactate/g tissue)**
M. Gastrocnemius pars interna			
10 min *post mortem*	25.2±5.0[c]	44.7±5.5[d]	91.4±10.1[b]
24 h *post mortem*	10.7±4.2[d]	62.6±10.5[c]	88.5±14.0[b]
72 h *post mortem*	13.9±4.5[d]	72.0±4.8[b]	99.9±11.5[b]
M. Iliofiburalis			
10 min *post mortem*	58.9±7.5[a]	47.2±4.5[d]	164.9±16.9[a]
24 h *post mortem*	42.6±6.5[b]	74.4±5.2[ab]	159.5±14.7[a]
72 h *post mortem*	40.0±14.9[b]	81.7±4.7[a]	169.3±20.8[a]
***p* values**			
Muscle effect	<.0001	<.0001	<.0001
Time effect	<.0001	<.0001	NS
Muscle x Time	NS	NS	NS

*Glycolytic potential
NS: not significant ($p > 0.05$).
[a-d] Means with the same superscript within same column do not differ significantly ($p \leq 0.05$).

Table 2.2

Means (±SD) for lipid content, α-tocopherol content, SOD, and catalase activity in two muscles of *rhea americana*.

Component	***M. Gastrocnemius pars interna***	***M. Iliofiburalis***	***p* value**
Total lipids (g/100 g tissue)	1.51±0.18	2.81±1.26	0.0292
Cholesterol (mg/100 g tissue)	56.0±12.8	81.6±16.5	0.0139
Fatty acids *(weight % total fatty acid methyl esters)*			
C14:0	0.36±0.11	0.43±0.11	NS
C16:0	14.59±2.20	19.35±3.61	0.0040
C18:0	14.02±1.15	13.78±1.10	NS
C16:1	1.98±0.57	2.39±0.76	NS
C18:1 n-9 cis	19.08±1.82	20.28±2.98	NS
C18:2 n-6	22.36±1.37	22.01±2.76	NS
C18:3 n-3	1.76±0.39	2.14±0.71	NS
C20:4 n-6	13.77±1.61	10.46±2.38	NS
C22:4 n-6	2.00±0.21	1.35±0.29	0.0002
C20:5 n-3	1.34±0.41	0.90±0.48	0.0012
C22:5 n-3	2.23±0.28	1.16±0.36	<.0001
C22:6 n-3	0.24±0.06	0.05±0.05	0.0005
Sum SFA*	29.88±2.06	34.44±2.89	0.0018
Sum MUFA*	24.70±2.06	26.09±3.34	NS
Sum PUFA*	45.15±3.03	39.09±5.62	0.0102
Sum n-3 PUFA	5.88±0.92	4.49±1.11	0.0012
Sum n-6 PUFA	39.28±2.39	34.60±5.17	0.0323
C16:0/C18:0	1.05±0.21	1.43±0,37	0.0226
n-6 PUFA /n-3 PUFA	6.79±0.85	8.10±2.31	NS
C18:2 n-6/C18:3 n-3	13.36±3.56	11.36±3.88	NS
PUFA/SFA	1.52±0.19	1.15±0.25	0.0025
α-tocopherol (µg/g tissue)	0.32±0.18	0.75±0.10	0.0002
SOD activity (U.)	1.77±0.47	2.04±0.35	NS
Catalase activity (nmol H_2O_2/min/mg protein)	6067.1±2031.6	4601.7±1626.4	0.0077

SOD: superoxide dismutase; U.: Units.
NS: not significant ($p > 0.05$).
* SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

Table 2.3

Means (±SD) for physicochemical quality parameters in two muscles of *rhea americana* during air-packaged storage.

	M. Gastrocnemius pars interna			***M. Iliofiburalis***			***p* values**		
	1 day	**3 days**	**5 days**	**1 day**	**3 days**	**5 days**	**Muscle effect**	**Time effect**	**Muscle x Time**
L*	36.2±1.8^{ab}	35.7±2.6^{ab}	32.0±3.3^{bc}	39.7±3.6^{a}	33.1±3.0^{bc}	28.9±2.6^{c}	NS	<.0001	0.0034
a*	16.5±0.8^{ab}	16.8±1.4^{ab}	15.7±1.4^{ab}	17.2±1.4^{a}	13.7±3.6^{b}	8.7±2.3^{c}	<.0001	<.0001	<.0001
b*	8.1±0.7^{a}	9.5±1.8^{a}	9.0±1.3^{a}	10.0±1.6^{a}	8.7±2.3^{a}	9.0±1.6^{a}	NS	NS	NS
$R_{630} - R_{580}$	14.6±1.2^{ab}	12.7±2.1^{b}	9.4±2.5^{c}	19.9±2.0^{a}	7.3±3.4^{c}	2.5±1.1^{d}	<.0001	<.0001	<.0001
Haeminic iron (µg/g tissue)	19.6±2.7^{a}	ND	ND	25.6±1.5^{b}	ND	ND	<.0001	NS	NS
Sensory Lightness	3.2±0.4^{a}	3.5±0.6^{a}	3.2±0.5^{a}	3.1±0.6^{a}	2.1±0.7^{b}	1.7±0.5^{b}	<.0001	0.0019	0.001
Sensory Redness	3.7±0.3^{a}	3.7±0.4^{a}	3.9±0.3^{a}	2.7±0.4^{b}	1.8±0.5^{c}	1.5±0.5^{c}	<.0001	0.002	0.0001
Sensory Aspect (Pleasure)	3.4±0.6^{ac}	3.4±0.6^{ac}	3.8±0.4^{a}	3.0±0.4^{c}	2.2±0.5^{b}	1.6±0.5^{b}	<.0001	0.0184	<.0001
Purchase intention %	75.0±17.7^{ab}	79.7±18.8^{ab}	87.5±14.8^{a}	54.7±16.3^{bc}	32.8±22.1^{c}	4.2±7.7^{d}	<.0001	0.0112	<.0001
TBA-RS (mg MDA/kg tissue)	1.1±0.4^{a}	1.6±0.4^{a}	2.2±0.9^{ab}	1.6±0.3^{a}	4.4±2.2^{b}	8.8±2.8^{c}	<.0001	<.0001	<.0001
Carbonyls (nmol/mg protein)	2.2±0.5^{a}	2.2±0.2^{a}	2.3±0.4^{a}	2.5±0.3^{ab}	3.1±0.8^{b}	3.9±0.7^{c}	<.0001	0.0013	0.0019

ND: not determined.
NS: not significant ($p > 0.05$).
[a-d] Means with the same superscript within same row do not differ significantly ($p \leq 0.05$).

Table 2.4

Means (±SD) for physicochemical quality parameters in two muscles of *rhea americana* during vacuum-packaged storage.

	M. Gastrocnemius pars interna				***M. Iliofiburalis***				***p* values**		
	7 days	**14 days**	**21 days**	**28 days**	**7 days**	**14 days**	**21 days**	**28 days**	**Muscle effect**	**Time effect**	**Muscle x Time**
L*	32.2±3.5^{ab}	28.9±3.4^{ab}	29.6±1.7^{ab}	30.4±2.8^{ab}	32.5±3.1^{a}	28.5±4.8^{ab}	28.3±2.3^{ab}	27.3±3.5^{a}	NS	0.0055	NS
a*	17.2±1.8^{a}	18.9±1.5^{a}	19.8±2.8^{a}	18.2±1.8^{a}	20.0±2.0^{a}	20.5±3.1^{a}	20.3±2.6^{a}	18.4±3.0^{a}	NS	NS	NS
b*	6.9±1.1^{a}	8.6±1.8^{ab}	10.3±2.3^{ab}	8.9±1.6^{ab}	10.2±2.5^{ab}	9.6±2.8^{ab}	10.8±2.7^{b}	10.7±1.9^{b}	0.0041	NS	NS
$R_{630} - R_{580}$	12.6±1.2^{ab}	12.0±1.9^{ab}	13.0±1.9^{ab}	12.6±1.4^{ab}	13.9±1.7^{b}	12.4±3.0^{ab}	12.1±1.7^{ab}	10.4±3.1^{a}	NS	NS	NS
TBA-RS (mg MDA/kg tissue)	1.1±0.1^{a}	1.2±0.2^{a}	1.3±0.3^{ab}	1.4±0.3^{ab}	1.8±0.2^{bc}	1.8±0.4^{bc}	2.1±0.4^{c}	1.8±0.3^{bc}	<.0001	NS	NS
Carbonyls (nmol/mg protein)	2.2±0.4^{a}	2.3±0.3^{a}	2.4±0.4^{a}	2.4±0.5^{a}	2.7±0.6^{a}	2.4±0.4^{a}	2.6±0.8^{a}	2.6±0.5^{a}	NS	NS	NS

NS: not significant ($p > 0.05$).
$^{a-c}$ Means with the same superscript within same row do not differ significantly ($p \leq 0.05$).

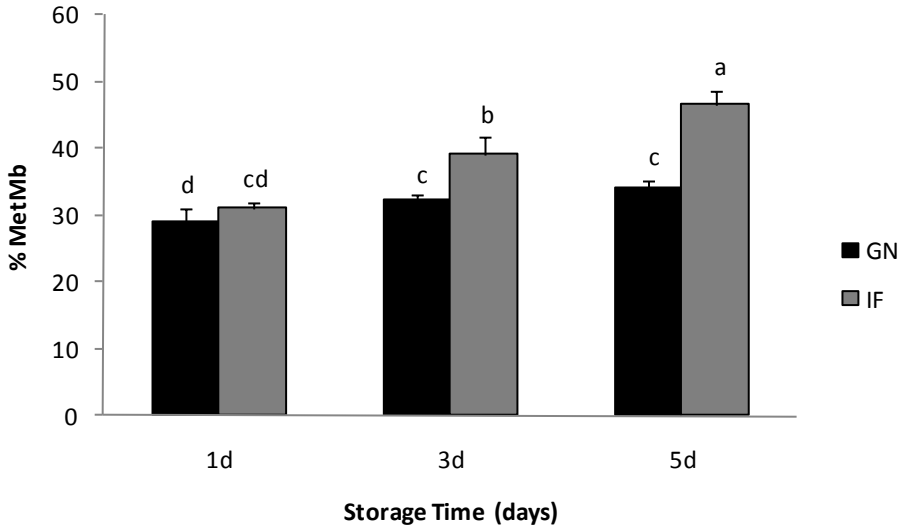

Figure 2.2. Percentage of metmyoglobin (%MetMb) in *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF) of *rhea americana* during air-packaged storage.
[a-d] Means with different superscript differ significantly ($p \leq 0.05$).
1d: 24 h *post mortem*; 3d: three days *post mortem*; 5d: five days *post mortem*;

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Chapter 3

Histological and ultrastructural characterization of *Rhea americana* muscles

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3.1. ABSTRACT

Morphometrical, histochemical and ultrastructural characteristics of rhea muscles were investigated in limb *M. Gastrocnemius pars interna* and *M. Iliofiburalis*. The fibres were larger and the extracellular area was superior in GN than in IF muscle, which presented maximum average diameter of 55 μm. Only one type of fibre was found in rhea muscles. Limb muscles were acid-labile and alkali-stable to m-ATPase, presented moderate to high SDH activity and high glycogen content. The ultrastructural observation of rhea myofibrils showed contracted and stretched areas, as well as abundant glycogen and numerous mitochondria, mainly in IF muscle.

Keywords: Rhea muscles; m-ATPase; SDH; glycogen; mitochondria; fibre type.

3.2. INTRODUCTION

Rhea americana is a flightless or running bird native from South America (Argentina, Bolivia, Brazil, Paraguay and Uruguay) that belongs the ratite group. Ratites have developed the leg muscles and the wings were atrophied. Their ability to run at high speeds is due to their highly specialized pelvic limb musculature (Abourachid, 2001). Rhea produces red meat, with similar concentration of myoglobin, lesser amount of fat, lower cholesterol and relatively high polyunsaturated fatty acids (Filgueras et al., 2010; Sales et al., 1999; Terevinto et al., 2010) compared to beef. However, in a previously study on rhea limb *M. Gastrocnemius* and *M. Iliofiburalis* it was demonstrated that, at the same time rhea meat presented advantages due its nutritional value, it also showed high oxidation instability during air-packaging storage (Filgueras et al., 2010). One explanation was the high haeminic iron concentration presented in rhea muscles, which acts as a pro-oxidant, increasing the speed of oxidation's reactions. On the other hand, high concentration of glycogen immediately after slaughter and residual glycogen after 24 h *post mortem* was also observed via biochemical analysis, mainly in *M. Iliofiburalis*, which was very instable when exposed to oxygen. This high glycogen content and, consequently, the fibre types of rhea muscles could be associated to the colour, lipid, and protein instability of meat during storage, but more studies are necessary to determine the possible role of glycogen concentration after rigor mortis and oxidative process developments.

A characteristic of skeletal muscle is its diversity, consisting of different kinds of fibres which moreover, vary within themselves (Pette & Staron, 1990). There are marked differences in fibre type composition of different muscles, both within and between animals, which may influence meat quality and depend on factors such as body location, age, weight, and breed (Cassens & Cooper, 1971; Essen-Gustavsson, 1995). Muscle fibres are the basic unit for muscle activity, containing enzymes that convert chemical energy into mechanical work and specific proteins that form myofibrils. During the neonatal period, muscle fibres acquire metabolic and functional characteristics related to the locomotory or postural function of the muscle (Lawrie, 1998; Velotto & Crasto, 2004).

Fibres may be classified according to contractile and/or metabolic properties. Among the numerous histochemical classifications, the most widely accepted is based on differences in the acid and alkaline stability of the myofibrillar ATPase (m-ATPase) and succinic dehydrogenase (SDH) activity. The slow-twitch oxidative (SO) or type I fibres present acid-stable m-ATPase, alkali-labile m-ATPase and oxidative metabolism (i.e., strong mitochondrial SDH activity). Fast-twitch glycolytic oxidative (FOG) or type IIA fibres present intermediate metabolism, i.e. acid-labile, alkali-stable m-ATPase and moderate SDH activity. Finally, fast-twitch glycolytic (FG) or type IIB fibres are acid-labile, alkali-stable m-ATPase and present low SDH activity (Brooke & Kaiser, 1970; Peter, Barnard, Edgerton, Gillespie, & Stemple, 1972).

The fibre types are important in meat animals because they play a key role in meat quality Differences in metabolism between the various fiber types influence *post mortem* metabolism, ultimate pH, colour, water-holding capacity, and tenderness (Fernandez, Meunier-Salun, & Ecolan, 1994; Hood, 1980; Klont, Brocks, & Eikelenboom, 1998; Monin & Ouali, 1992; Xiong, Cantor, Pescator, Blancherd, & Straw, 1993). No data about fibre type of rhea muscles were found in the literature. So, the aims of this study were to characterize the fibre types of two limb muscles of *rhea americana* and associate the results with meat quality.

3.3. MATERIAL AND METHODS

3.3.1. Sample preparation

Muscles *Gastrocnemius pars interna* (GN) and *Iliofiburalis* (IF) were obtained from 12-month-old Rhea Americana. The animals (n=8) were reared on a semi-intensive farm system in Provins (France) and slaughtered at the experimental slaughterhouse of the INRA Research Centre of Theix (France) Eight 12-month old animals were rendered unconscious by a sharp blow to the head then immediately killed by severing the carotid arteries in the neck at the experimental slaughterhouse of the INRA Research Centre of Theix (France). This is an approved method of killing ostriches in France. The carcasses were maintained at room temperature (15 °C) for 1 hour and then chilled at 4 °C

overnight. Sample collection was performed 15 minutes and 24 h *post mortem* and prepared for histochemistry and ultrastructural observations.

3.3.2. Optical microscopy

3.3.2.1. Morphology

For each collection time, samples were frozen in cooled isopentane chilled with liquid nitrogen (-160 °C). Thin transverse serial sections (10 µm thick) were sliced at -20 °C using a cryostat (Microm, HM 560), mounted on slides and air-dried (20 °C). Sections were stained using Hematoxylin Eosin Safran coloration (Sheehan & Hrapchak, 1980) to visualise overall structure.

3.3.2.2. Fibre type

Delineation of the different types of fibres on serial cross-sections of muscles was based on the combination of the pH sensitivity of myofibrillar ATPase after acid pre-incubation at pH 4.6 and basic pre-incubation at pH 10.4 (Guth & Samaha, 1969), and succinate dehydrogenase (SDH) activity (Gautier, 1969). Myofibrillar ATPasic activity allows to distinct fibres with slow and fast contraction rate. ATPase of fast fibres is stable in basic environment, and labile in acid one. The reverse situation is true for slow fibres. SDH activity reveals the oxidative metabolism. The combination of these two stainings enabled to classify the different fibres types as slow oxidative (SO), fast oxidative glycolytic (FOG) or fast glycolytic (FG) (Peter et al., 1972). The percentage of each type of fibre was measured in two randomly selected areas on serial sections. An average of 200 muscle cells were analysed in each serial section.

3.3.2.3. Glycogen staining

Periodic acid Schiff (PAS) was used to stain glycogen content on cross sections of muscles at 15 min and 24 h *post mortem*, according to Pearse (1968).

3.3.2.4. Observation and images acquisition

Observations were performed using an Olympus motorized BX61 transmission white field/fluorescence microscope. Images were acquired with an Olympus cooled digital camera DP-71 coupled to Cell-F software (Olympus Soft Imaging Solutions). In order to make comparisons between muscles, the observations were all performed using the 10x or 20x objectives. Image analysis software (ImageJ) was used to evaluate the fibre density on transversal cuts (number of fibre profiles per mm2), the area of individual fibres (µm2), the perimeter of fibres (µm), the maximum and minimum diameter of fibres (µm), the circularity, and the extracellular area between fibres (µm2). For each muscle, not less than 1700 fibres were counted in the four cross-sections. Only fibre profiles entirely within the counting frame were counted and analysed.

3.3.3. Electron microscopy

A piece of muscle, about 10x3x3 mm, taken from the heart of the sample, was fixed overnight at 4° C by immersion in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer, pH 7.2, taking into account the muscle fibre orientation. Small blocks (1 to 3 mm3) were post-fixed in 1 % osmium tetraoxyde in 0.1M sodium cacodylate buffer for 1 hour at ambient temperature. The blocks were dehydrated through a graded series of ethanol and embedded in epoxy resin (TAAB, Eurobio France). Semi-thin sections (1.5 µm) of transversal and longitudinal fibres were stained with toluidine blue and observed with an optical microscope. Areas presenting muscle fibres were selected for ultrastructure observations. Ultra-thin sections (90 nm) were stained with uranyl acetate and lead citrate (Reynolds, 1963) and observed with a transmission electron microscope (Itachi HM 7650) using an 80 KV acceleration voltage. Micrographs were made using a Hamamatsu AMT numeric camera system coupled with the microscope. The sample preparation was done at the Electron Microscopy laboratory of INRA de Theix (France) and observations at the Imaging Cellular Center for Health (CICS) laboratory located on the university campus of Clermont-Ferrand (France). Ultrastructural observations were performed on half of the animals used in this study.

3.3.4. Statistical analysis

Morphometrical data acquired by image analysis was expressed as mean ± standard error of the mean. Quantification of myofibrillar mass area, perimeter, diameters, spaces between myofibrillar mass, and fibre density were analysed under the Statistical Analysis System (1988) by one-way analysis of variance (ANOVA) using the general linear model procedure and the unpaired Student t-test to determine the statistical significance levels between muscles.

3.4. RESULTS

3.4.1. Morphology

The results of morphometrical parameters estimated in rhea muscles are presented in Table 3.1. The fibres were 1.4 times larger in GN muscle than in IF muscle, with a density of fibres per mm^2 of 43.4 and 67.8 in GN and IF muscle, respectively. Besides, the extracellular area in GN was superior than in IF muscle, but in both muscles it did not reach 4% of total area. Sales (1996) also reported significant differences between *M. Gastrocnemius pars externa* and *M. Iliofiburalis* of ostrich. The average diameters reported by this author were 57.5 and 49.5, for GN and IF, respectively, and the fibre diameter was not correlated to tenderness (Warner-Bratzler shear measurement).

3.4.2. Fibre types

Fibre types were classified according to the nomenclature of Peter et al. (1972). We used the m-ATPase histochemical assay to differentiate between fast and slow fibres. Moreover, the SDH technique was used to separate oxidative from anaerobic fibres. Succinic dehydrogenase (SDH), a constitutive molecule of complex II of the mitochondrial respiratory chain, play an important role at high respiration rates, thus the activity of this enzyme has been considered a good indicator of the mitochondrial oxidative capacity (Linderholm et al., 1990; Chalmers et al., 1992; Beal et al., 1993).

Slow oxidative fibres (SO) which generally present low alkaline and high acidic m-ATPase stability and great SDH staining were not found in serial cross-sections of rhea muscles. Both *M. Gastrocnemius pars interna* and *M. Iliofiburalis* of rhea presented a high homogeneity of fibres. Curiously, only one type of fibre was found in rhea muscles. As shown in figure 3.1, rhea limb muscles analyzed in this work were acid-labile and alkali-stable to m-ATPase. In alkaline pre-incubation, fibres of both muscles were dark stained and presented very homogeneity in comparison with M. Gastrocnemius of duck, used as control in this study for its heterogeneity in m-ATPase activity. In addition, muscles also presented very high homogeneity and moderate to high SDH activity (Fig. 3.2 A, B), indicating important mitochondrial oxidative capacity of muscle fibres. However, the blue staining is more spread on the whole cell in IF muscle.

Finally, study of the concentration of glycogen highlighted a distinguished difference between the two muscles. The PAS staining revealed important glycogen concentrations in both rhea muscles, but the IF muscle showed a markedly higher concentration when compared to GN muscle (Fig. 3.2 C, D). Thus, the muscles GN and IF of rhea can be classified as fast-twitch contracting muscle with intermediate metabolism, i.e. FOG fibres (fast-twitch glycolytic oxidative), because they presented both oxidative apparatus and high glycogen content and only alkalin-stable m-ATPase fibres, demonstrating the fast contracting ability of muscles.

3.4.3. Ultrastructural level

The figure 3.3A shows micrographs from GN muscle. Contraction areas of myofibrils are followed by stretched areas. This observation was found in both muscles. Suh a high contraction is rarely found in poultry or pigs muscles, contrarily to beef muscles where such heterogeneity in morphology is present in meat too rapidely chilled while ATP content is still high. In our case, pH was under 6 after 1.5 hour and the muscle temperature above 25°C, far from cold shortening risk (Filgueras et al., 2010).

The figure 3.3 B shows glycogen granules, which were very abundant in muscles cells, especially in IF muscle (see Fig. 3.2). The figures 3.3C and 3.3D show the mitochondria repartition in GN (Fig. 3.3C) and IF muscle (Fig. 3.3D). Both muscles had numerous mitochondria, but in GN muscles the mitochondria were located closed to the

sarcolemme, structure which is generally present in muscle cell. On the contrary, the IF muscle presented additional mitochondria located between myofibrils, which is rarely even never found. This observation confirms the pattern of the blue staining of SDH, which reaction was spread over the muscle cells.

3.5. DISCUSSION

Our study showed that the GN and IF muscles presented one type of muscle fiber. At the ultrastructural level this information was completed by the repartition of mitochondria which differed between muscles. The comparison with studies on ostrich Velotto & Castro (2004) showed that *M. Gastrocnemius pars externa* (extensor of the foot) presented the three types of fibres: FG, in higher proportion (36%), and FOG and SO, in lesser proportion (33 and 31%, respectively). However, in the *M. Tibialis cranialis* and *M. Fiburalis longus* these authors reported the absence of FG fibres.

According to Patak & Baldwin (1993), although the fibre types were not easily distinguished and appeared continuously variable in emu muscles, muscles presenting more FG fibres generally presented large FG fibres and small FOG fibres, while muscles presenting the three types of fibres had large FG and FOG, and small SO fibres. The *Gastrocnemius lateralis*, *G. intermedius caudalis* and *G. intermedius medialis* muscles of emu presented 55 to 58% of FG fibres, and 42 to 45% of FOG fibres (Patak & Baldwin, 1993). As reviewed by Klont et al. (1998), an important point when analysing muscle fibres type composition and its relation with meat quality are the structural differences associated with different fibre types and the variation in fibre type within muscles. Most research showed that there is an inverse relation between fibre diameter and oxidative capacity; the FOG fibres generally presenting intermediate size (Cassens & Cooper, 1971; Rosser, Norris, & Nemeth, 1992). In addition, the SO and FOG fibres have usually greater lipid and myoglobin content than FG fibres (Essen-Gustavsson, Karlström, & Lundström, 1992). For example, muscles involved in posture are redder and more oxidatives than those involved in movements, which are whiter and more glycolytic (Henckel, 1995; Klont et al., 1998).

The variation in muscle oxidative, glycolytic and/or contractile properties can also be assessed by biochemical techniques, since the concentration, activities and or ratios of different enzymes for contraction and energy metabolism differ in the various types of muscle (Kiessling & Hansson, 1983; Klont et al., 1998; Pette & Staron, 1990). In this study, the hitochemical results were consistent with previously biochemical assays on glycogen concentration of GN and IF muscles of rhea immediately after slaughter and 24 h *post mortem* (Filgueras et al., 2010). Rhea GN and IF muscles presented fast pH decline and normal ultimate pH (between 5.4 and 5.6) at 24 h *post mortem*, but both muscles presented high residual glycogen after rigor mortis installation, mainly IF muscle (GN=10.7 and IF=42.6 μmol/g tissue) (Filgueras et al., 2010). Glycolytic FG and FOG fibres have higher glycogen concentration and are metabolically better equipped for anaerobic glycogen utilization than SO fibres. Thus, in general, glycolysis and onset of rigor mortis are faster in FG and FOG than in SO fibres. It was well demonstrated in chickens by Xiong et al. (1993) that reported a faster and more pronounced (ultimate pH lower) pH decline in glycolytic white muscle (e.g. pectoral) than in oxidative red muscles of chicken thigh. Similarly, Addis (1986) demonstrated that in turkeys the *post mortem* pH decline was faster in white (SO) than in red (FG) muscles. This phenomenon may be explained by the fact that muscles rich in SO fibres have lower ATPase activity, which slows down the speed of the pH decline (Lefaucher, LeDividich, Mourot, Monin, Escolan, & Krauss, 1991).

However, rhea muscles presented the two characteristics: oxidative and glycolytic fibres. Variation in meat quality was related to heterogeneity in glycogen depletion between different fibre types in pig (Karlson, Enfält, Essen-Gustavsson, Lundström, Rydhmer, & Stern, 1993; Fernandez et al. 1994). Concerning colour stability of meat and fibre characteristics, studies have demonstrated that oxygen consumption in muscles seems to be inversely correlated to stability of colour (Hood, 1980; Klont et al., 1998), and muscle fibre type was the major factor controlling the rate of discolouration of beef exposed to oxygen (Hood, 1980). Differences in oxygen consumption rate and myoglobin autoxidation rate explained better the variation in colour stability between different muscles than the enzymatic ferrimyoglobin reduction did (Renerre & Labas, 1987; Klont et al., 1998). Higher proportion of oxidative fibres coincide with higher concentration of mitochondria in muscles, and after aerobic

exposure of the meat surface, intact mitochondria might compete with myoglobin for the uptake of oxygen thereby reducing the depth of the bright-red oxymyoglobin layer (Monin & Ouali, 1992). It can explain the higher colour instability and consequently lipid and protein oxidation of rhea IF muscle during air-packaging storage because metmyoglobin formation is related to lipid oxidation (Chan, Faustman, Yin, & Decker, 1997; Faustman, Liebler, McClure, & Sun, 1999). In addition, the interactions between lipids and proteins have a significant effect on the progress of oxidative reactions in meats (Kamin-Belsky, Brillon, Arav, & Shaklai, 1996; Liu & Xiong, 2000; Santé-Lhoutellier, Astruc, Marinova, Grève, & Gatellier, 2008). In rhea, the residual glycogen after 24 h *post mortem* in IF muscle was 4 times greater than in GN muscle, and IF was darker, with high discolouration rate, high content of metmyoglobin and considered inacceptable for sensorial jury after 3 days of display in comparison to GN muscle, which was more stable and presented more red-bright colour (Filgueras et al., 2010).

3.6. CONCLUSION

Both muscles of rhea limb (*M. Gastrocnemius pars interna* and *M. Iliofiburalis*) presented only one type of fibres, i.e. fast-twitch oxidative and glycolytic FOG fibres. The homogeneity of fibres was evident after m-ATPase, SDH and glycogen staining reactions. The high mitochondrial oxidative capacity of rhea muscles associated to the high concentration of residual glycogen, mainly in IF muscle, may be involved in oxidative instability of meat exposed to oxygen, but further investigations are necessary to verify this hypothesis.

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Table 3.1.
Morphometrical parameters of rhea muscle fibres (means ± SD).

Parameter	Rhea muscle		*P value*
	M. Gastrocnemius pars interna	*M. Iliofiburalis*	
Density of fibres (mm^2)	43.42 ± 10.91	67.81 ± 16.04	*<.0001*
Fibre area (μm^2)	2330.0 ± 507.3	1584.9 ± 324.9	*<.0001*
Perimeter (µm)	218.1 ± 31.97	187.1 ± 28.21	*<.0001*
Maximum diameter (µm)	69.26 ± 10.26	55.61 ± 7.48	*<.0001*
Minimum diameter (µm)	41.92 ± 4.57	35.39 ± 4.67	*<.0001*
Extracellular space area (μm^2)	5472.2 ± 863.4	4543.1 ± 1145.1	*0.0002*
Extracellular spaces (%)	3.81 ± 0.6	3.17 ± 0.8	*0.0002*

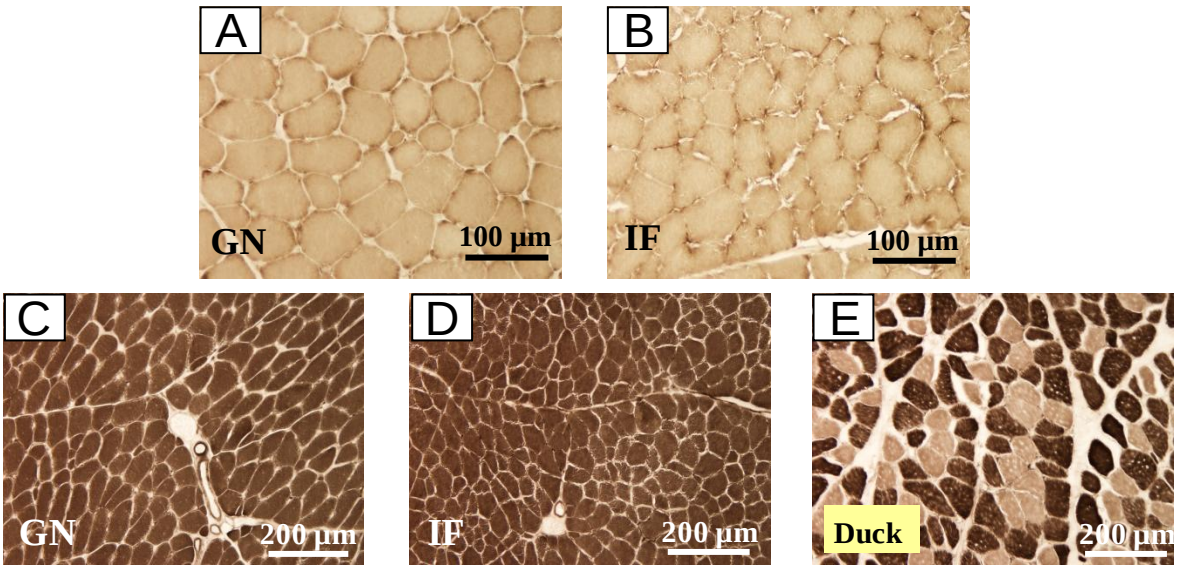

Figure 3.1: Contractile characterization of muscle fibres using ATPase activity after acid pre incubation on *Gastrocnemius pars interna* (GN) and *Iliofiburalis* (IF) muscles (A, B) and basic pre-incubation (pH = 10.4) (C, D). *Gastrocnemius* muscle of duck was used as control (E).

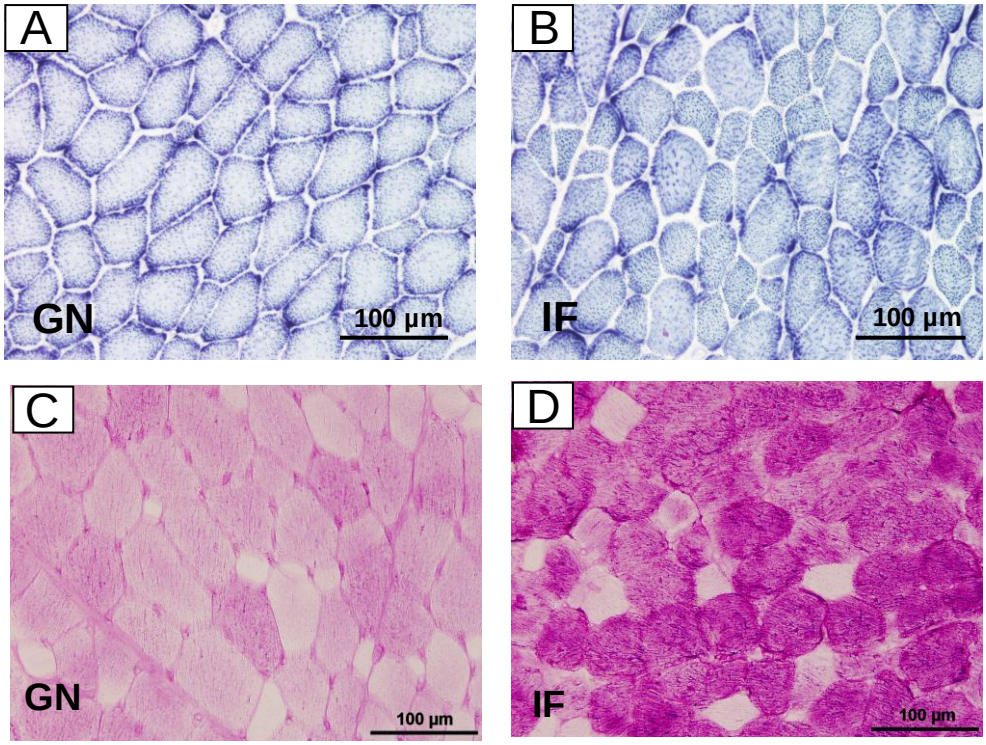

Figure 3.2: Oxidative metabolism characterization using SDH activity on *Gastrocnemius pars interna* (GN) and *Iliofiburalis* (IF) muscles (A, B) and Glycogen characterization using PAS reaction on *Gastrocnemius pars interna* (GN) and *Iliofiburalis* (IF) (C, D).

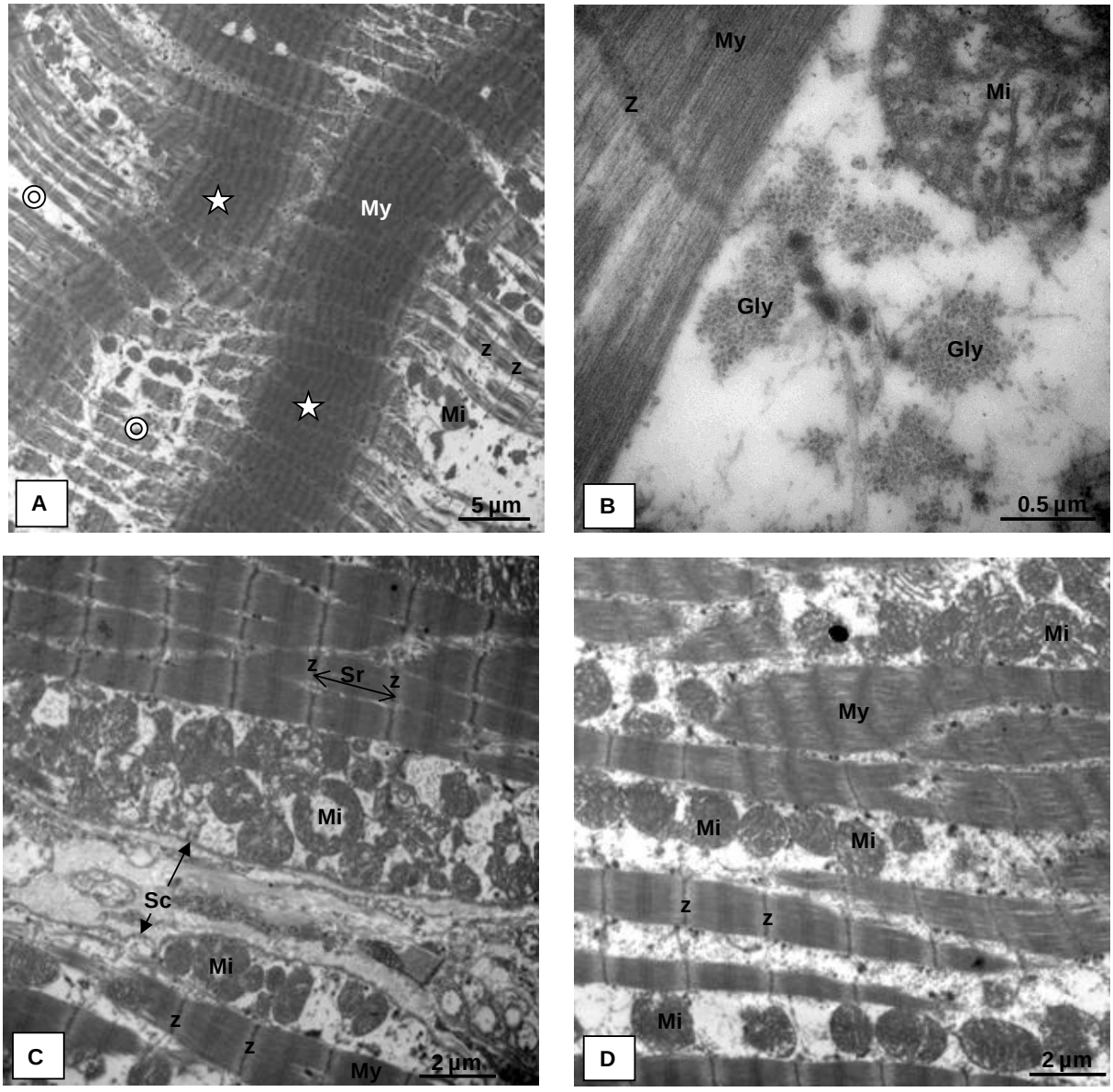

Figure 3.3. Ultrastructural characteristics of rhea muscles.
A: Exemple of musle morphology in tight rhea muscle. Contracted myofibrils (stars) often followed by stretch myofibrils (circles).
B: Glycogen granules (Gly) are visibles between the myofibrils (My).
C: GN muscle: mitochondria (Mi) are generally located in the subsarcolemmal space of the cells.
D: IF muscle: A high proportion of the mitochondria is located in between the myofibrils

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Chapter 4

Effect of frozen storage duration and cooking on physical and oxidative changes of rhea meat

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4.1. ABSTRACT

This study was conducted to evaluate the effect of frozen storage time (30, 60, 90 or 180 days) and cooking (100 °C, 30 min) on physical characteristics and oxidative stability of *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF) of *rhea americana*. Physical parameters measured included thawing and cooking loss, colour attributes, while oxidation was assessed by determining the TBA-RS, carbonyl and aromatic amino acid content. Prolonged frozen storage of rhea meat decreased lightness (L*), yellowness (b*), and increased the discoloration parameter hue angle. During storage, muscle IF was more prone to lipid and metmyoglobin oxidation than muscle GN. Cooking loss declined with the increase of storage time and was higher in GN than in IF muscle. With cooking, TBA-RS, carbonyl content, and aromatic amino acids (phenylalanine, tyrosine, and tryptophan) were highly affected, but the extent of oxidation ranged according to muscle and duration of frozen storage.

Keywords: Rhea meat; frozen storage; colour; TBA-RS; protein oxidation.

4.2. INTRODUCTION

The number of studies on ratite meat production and products has increased in the last years and South Africa remains the major supplier of ratites products with their production of 214,000 ostriches over the 300,000 produced in the world. Most of the studies focused on production yield (Morris et al., 1995ab) and meat quality, highlighting its chemical composition and nutritional value (Berge, Lepetit, Renerre, & Touraille, 1997; Pegg, Amarowicz, & Code, 2006; Sales, 1996 and 1998; Sales, Navarro, Bellis, Manero, Lizurume, & Martella, 1998; Sales, et al., 1999, Filgueras et al, 2010). Ratites (ostrich, emu and rhea) are sources of proteins and minerals for human diet and their meats have been perceived and marketed as healthy due to their low intermuscular fat, and cholesterol content (Fisher, Hoffman, & Mellet, 2000; Paleari, Corsico, & Beretta, 1995; Sales et al., 1999; Saadoun et al., 2008). In addition, intramuscular ratite fat presents a high content of polyunsaturated fatty acids (Girolami, Marsico, D'Andrea, Braghieri, Napolitano, & Cifuni, 2003; Sales, 1998; Sales et al., 1999). Not only the amount of fat, but also the fat quality is important for the health. The saturated fatty (SF) acids contribute to heart disease by raising plasma low density lipoprotein cholesterol, while polyunsaturated fatty acids (PUFA) decrease the risk of heart disease by lowing it (Aaslyng, 2009). Moreover a high level of iron and haem iron is found in the muscles (Lombardi-Boccia, Martínez-Domínguez, Aguzzi, & Rincón-León, 2002; Ramos, Cabrera, Del-Puerto, & Saadoun, 2009), which confers to meat a red colour and similar taste to beef.

However, at the same time the composition of ratite's meat presents advantages and health benefits, it can lead to an increase in susceptibility to oxidation, deteriorating quickly the meat quality, because lipid composition is rich in Polyinsatured fatty acids (Filgueras et al., 2010). Storage and cooking are indispensable to achieve a safe and palatable product, but depending on the conditions applied, they can enhance the development of oxidation processes, decreasing meat quality and meat nutritional value. By its production localized in specific country (Ostrich inSouth Africa, Emu inAustralia, Nandou inBrazil, Argentine and Uruguay), the exportations of meat products require generally long storage. Frozen allow extended storage from a sanitary point of view but do not prevent oxidation processes (Stika et al., 2010). Oxidative processes are complex

reactions initiated from meat pigments, different classes of lipids, and proteins. A variety of oxidation products are formed. Lipid oxidation is known as one of the major causes of deterioration in the quality of meat and meat products and oxidation can occur in either the stored triglycerides or the tissue phospholipids. Haem pigments have been implicated as the major pro-oxidants in tissue lipid oxidation (Love, 1983; Renerre & Labadie, 1993). Oxidation of lipids is accentuated in the immediate post-slaughter period, during handling, processing, storage and cooking, leading to discoloration, drip losses, off-odor and off-flavor development, texture defects and the production of potentially toxic compounds (Morrissey, Sheehy, Galvin, & Kerry, 1998; Richards, Modra, & Li, 2002;). Lipid oxidation and myoglobin oxidation in meat are coupled and both reactions appear capable of influencing each other (Faustman, Yin, & Nadeau, 1992; Yin, & Faustman, 1993). The oxidation of oxymyoglobin results in the production of metmyoglobin and H2O2 necessary to induce lipid oxidation (Chan, Faustman, Yin, & Decker, 1997). On the other hand, aldehyde lipid oxidation products alter myoglobin redox stability, resulting in the promoted oxidation of oxymyoglobin and the formation of adduct with myoglobin through covalent modification (Linch & Faustman, 2000).

Besides lipid and myoglobin oxidation, protein oxidation is also responsible for many biological modifications (Decker, Xiong, Calvert, Crum, & Blanchart, 1993) such as protein fragmentation or aggregation, decrease in protein solubility and decrease in bioavailability of amino acids (Santé-Lhoutellier, Astruc, Marinova, Grève, Gatellier, 2008). Active oxygen species attack the side chain of basic amino acids (lysine, histidine, arginine) and can convert them into carbonyl derivatives. These carbonyl groups can react with free amino groups to form amide bonds. Oxidative processes on proteins can also decrease thiol groups by forming disulfide bridges. Finally, aromatic amino acids can also be oxidized (Martinaud, Mercier, Marinova, Tassy, Gatellier, & Renerre, 1997; Morzel, Gatellier, Sayd, Renerre, & Laville, 2006; Santé-Lhoutellier, Aubry, & Gatellier, 2007; Santé-Lhoutellier et al., 2008).

Freezing is a widely accepted preservation method used to store meat for relatively long periods of time. Utilizing frozen products rather than chilled offers the advantages of increased storage time, greater flexibility in inventory and greater product control (Pietrasik & Janz, 2009). However, freezing and frozen storage of meat can affect the structural and chemical properties of muscle foods, and influence meat

quality attributes such as thawing loss, colour and tenderness (Farouk & Swan, 1998; Honikel, Kim, Hamm, & Roncales, 1986). It is commonly known that, during the freezing process, intracellular juice is expelled by osmosis to the extracellular space forming ice crystals that later cause juice loss from meat during thawing (Farouk & Swan, 1998). These types of effects can strongly influence the quality attributes of meat and meat products and consumer acceptance (Pietrasik & Janz, 2009).

The aim of this study was to evaluate the effect of frozen storage and cooking on physical parameters and oxidation stability of two rhea muscles (*M. Gastrocnemius pars interna* and *M. Iliofiburalis*). Meat was maintained frozen at - 20 °C for up to 180 days, and cooking was conducted at 100 °C for 30 min.

4.3. MATERIAL AND METHODS

4.3.1. Animals and sampling

The experiment was carried out with rhea *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF), situated in the leg and thigh of rhea carcass, respectively. Eight 12-month-old animals (*rhea americana*) were knocked senseless and slaughtered at the experimental slaughterhouse of the INRA Research Centre of Theix (France). Carcasses were maintained at room temperature (15 °C) for 1 hour and then chilled at 4 °C overnight. The GN and IF muscles were removed from carcasses at 24 h *post mortem* and cut into five 2 cm-thick steaks of similar weigh (~60 g), which represented muscle samples at 0, 30, 60, 90, and 180 days of storage. Control samples (0 day) were transported to the laboratory for the analyses of physical parameters, then frozen and stored at -80 °C for posterior determination of lipid and protein oxidations. The additionally samples, subjected to freezing, were immediately vacuum-packaged, frozen at -20 °C and stored at -20 °C in a freezer with temperature monitored for until 180 days. Prior their evaluation, samples were removed from frozen storage and held under darkness for 24 h at 4 °C.

4.3.2. Thawing loss

Frozen samples of muscles were removed from their packaging, weighed, placed in a tray and thawed at 4 °C for 24 hours. The thawing loss (TL) was determined post-cooling by measurement of frozen and thawed weights of steaks. The results were expressed as the average proportion (% TL = [(frozen weight - thawed weight)/frozen weight] x 100).

4.3.3. Meat cooking

Samples in triplicates (5g) were sealed in polypropylene test tubes (inner diameter = 10 mm and thickness = 1 mm) and heated at 100 °C, in a digital temperature-controlled dry bath (Block–heater, Stuart–Scientific) for 30 min. This treatment reflected meat cooking in an oven for which similar temperatures can be reached between meat core and meat surface. The centre temperature of the samples was measured with a digital thermometer fitted with a thin temperature probe. After cooking treatment, samples were cooled at room temperature for 15 min to reach 18-20 °C, and then frozen at -80 °C until use.

4.3.4. Cooking loss

Cooking loss (CL) was calculated as the ratio between the post-cooking and the pre-cooking weight, and expressed as a percentage (% CL = [(thawed weight - cooked weight)/thawed weight] x 100).

4.3.5. Colour

Visible reflectance spectra (from 360 to 760 nm) were determined with an Uvikon 933 (Kontron) spectrophotometer equipped with an integrating sphere. Reflectance spectra were collected from a 2° viewing angle and with illuminant D65 (Daylight) lighting conditions. Colour coordinates were expressed as lightness (L*), redness (a*) and yellowness (b*). Oxygenation index (ΔR) was determined by reflectance difference between 630 nm and 580 nm (Renerre, 2000). The metmyoglobin

percentage (% MetMb) at the meat surface was determined by the method of Krzywicki (1979).

4.3.6. Lipid oxidation

Lipid oxidation was measured by the thiobarbituric acid-reactive substances (TBA-RS) method, according to Lynch & Frei (1993) and modified by Mercier, Gatellier, Viau, Remignon, and Renerre (1998). Muscle samples (1 g) were homogenized with 10 ml KCl 0.15 M + BHT 0.1 mM with a Polytron (1 min, medium speed). Homogenates (0.5 mL) were incubated with 1% (w/v) 2-thiobarbituric acid in 50 mM NaOH (0.25 mL) and 2.8% (w/v) trichloroacetic acid (0.25 mL) in a boiling water bath for 10 min. After cooling at room temperature for 20 min, the pink chromogen was extracted with n-butanol (2 mL) and its absorbance measured at 535 nm against a blank of n-butanol. TBA-RS concentrations were calculated using 1,1,3,3 tetrathoxypropane (0-0.8 µM) as standard. Results were expressed as mg of MDA per kg of meat (TBA units).

4.3.7. Carbonyl content

Muscle samples (1 g) were homogenized in 10 ml KCl 0.15 M + BHT 0.1 mM with a Polytron (1 min, medium speed). Proteins were treated with DNPH using the method of Oliver, Alin, Moerman, Goldstein, & Stadtman (1987) modified by Mercier et al. (1998). Results were expressed as nanomoles of DNPH fixed per milligram of protein.

4.3.8. Aromatic amino acids content

The aromatic amino acids (phenylalanine, tyrosine and tryptophan) were determined according to the method reported by Gatellier, Kondjoyan, Portanguen, Grève, Yoon and Santé-Lhoutellier (2009a). All measurements were performed in duplicate and values expressed as percentage of amino acid in fresh tissue (g/100 g meat).

4.3.9. Statistical analysis

Analysis of variance (ANOVA) was performed using the General Linear Model (GLM) procedure of SAS system. The linear model included fixed effects of muscle, storage time, and heating (for TBA-RS and protein oxidation). When significant effects were encountered, least squares means were compared using LSMEANS with PDIFF option and TUKEY adjustment.

4.4. RESULTS AND DISCUSSION

4.4.1. Thawing and cooking losses

The ability of meat to retain moisture (water-holding capacity) is the most important quality aspect of raw products and moreover for frozen meat, since it affects consumer acceptance and final weight of the product (Huff-Lonergan & Lonergan, 2005). In both muscles the thawing loss increased with the increase of storage duration ($p \leq 0.001$; Table 4.1). After 6 months of storage, the thawing loss averaged 7% and 8.4% in GN and IF muscles, respectively. In lamb or beef meat the thawing loss increased with frozen storage duration, but with a lesser extent (Boles & Swan, 2002a; Muela, Sañudo, Campo, Medel, & Beltrán, 2010). These formers reported 2.3% thawing loss after 1 month and up to 3.8% after 6 months, while in pork the thawing loss reached of 6-7% after 30 days (Mortensen, Andersen, Engelsen, & Bertram, 2006). Our study did not show any differences between muscles for this trait. Freezing, frozen storage and thawing cause cellular damage and can reduce meat quality due to the formation of ice crystals and resulting local increase in the salt concentration (Ballin & Lamersch, 2008; Smith, 1950;). The extent of the damage caused by freezing depends on the size of the ice crystals (Grujić, Petrović, Pikula, & Amidžić, 1993; Martino, Otero, Sanz, & Zaritzky, 1998; Mortensen et al., 2006). Slow freezing rates produce the largest crystals (Ballin & Lametsch, 2008; Devine, Graham, Lovatt, & Chrystall, 1995; Muela et al., 2010), which causes higher protein denaturation and leads to an increase of drip loss (Farouk & Swan, 1998). Prolonged freezer storage has been found to level out effects of freezing rate (Ngapo, Barbare, Reynolds, & Mawson, 1999), which is suggested to be a consequence of

recrystallisation of small crystals into bigger crystals during long-term freezer storage (Farouk, Wieliczko, & Merts, 2003).

Percentage of cooking loss was determined in a subsample (0 day, 30 days, and 180 days of storage). The cooking loss was higher in GN than in IF muscle and significant effects of muscle ($p \leq 0.001$) and storage time ($p \leq 0.05$) were observed. Other studies have reported that muscles differed in cooking losses (Cecchi, Huffman, Egbert, & Jones, 1988; Crouse, Cross, & Seidman, 1984; Jeremiah, Dugan, Aalhus, & Gibson, 2003). Jeremiah et al. (2003) reported that fat content in beef muscles was negatively related to total cooking loss, and fatter cuts presented lower thawing and cooking losses than cuts containing more moisture. In a previous study, we have observed that rhea GN muscle presented less total lipids than rhea IF muscle (Filgueras et al., 2010). It can explain the higher percentage of cooking loss in GN than in IF muscle of rhea.

In this study, cooking loss decreased as storage time increased. In GN muscle, cooking loss in control was significantly higher than cooking loss in samples frozen for 180 days. Boles & Swan (2002a) reported that frozen storage time significantly affected cook yield of beef inside rounds with the highest yield occurring after 5 weeks of frozen storage. In beef (Pietrasik & Janz, 2009) and in lamb (Muela et al., 2010), no significant effect on cooking loss was observed as a result of freezing and thawing.

Considering the total loss, both muscle and storage time had significant effects on total percentage ($p \leq 0.0001$). Total loss increased in both muscles with storage time. GN muscle of rhea stored for 180 days presented the highest percentage of loss found in this study.

4.4.2. Instrumental colour parameters

Storage time and muscle effect on colour of rhea meat are shown in Table 4.2. Muscle had no effect on lightness (L*), while storage time influences significantly the L* values of rhea meat. An interaction between muscle and storage time ($p \leq 0.05$) was observed. In control (0 day of storage) lightness of both muscles was significantly higher ($p \leq 0.0001$) than L* in frozen samples, which showed a darkening of the meat. It can be explained by the losses in water during thawing. After thawing, the cells damage and exudation leads to a meat less reflective than meat that was never frozen, and it

appears darker and less attractive than chilled meat (Jeremiah, 1981; Moore & Young, 1991), especially after long periods of frozen storage (Farouk & Swan, 1998; Moore & Young, 1991). On the contrary, Farouk et al. (2003) reported that the application of slowly freezing and thawing of beef leads to greater values of L* than the application of fast freezing and thawing. In general, freezing tends to reduce lightness (Moore & Young, 1991), especially after long periods of frozen storage (Moore & Young, 1991; Farouk & Swan, 1998). However, in our study, samples frozen for 180 days did not present significant lower L* values when compared with samples frozen for 30 days, even though L* values vary during storage time for both muscles (Table 4.2). Muela et al. (2010) have not either observed a decrease of lightness during frozen storage of lamb meat; a. nd Moore and Young (1991), and McKenna et al. (2005) reported that the L* of fresh and frozen lamb chops was more stable during chill storage than the other colour parameters., and Zhu and Brewer (1998), Hansen, Juncher, Henckel, Karlsson, Bertelsen, & Skibsted (2004), and Berruga, Vergara, and Gallego (2005) reported similar patterns in the stability of L* in lamb.

Redness (a*) is the most important color parameter to evaluate meat oxidation because the transformation of oxymyoglobin (bright red color) to metmyoglobin (brown) decreases the redness a* and makes the meat unacceptable for the consumer (Renerre, 2000). In this study, significant effect of storage time was observed. However, when considering just the period of frozen storage, redness was stable from 30 to 180 days of storage at -20 °C ($p > 0.05$), indicating that freezing and thawing leaded to a decrease in redness of rhea meat which remained quite stable until 6 months. Contrarily, according to Fernández, Sanz, Molina-García, & Otero (2007) and Stika, Xiong, Suman, Blanchard, and Mood (2007), meat stored for long periods presents lower a* value than meat stored for short periods, probably because frozen storage reduces the activity of metmyoglobin-reducing enzymes. The reduction in metmyoglobin-reducing enzymes activity increases with the increase of storage time (Faraouk & Swan, 1998). In lamb, Muela et al. (2010) reported that frozen storage time had a significant effect on a*, but the evolution was not linear and redness values oscillated during storage, being significantly higher in meat stored frozen for 3 months than meat stored for 1 or 6 months. Redness in control samples were significantly higher than redness in samples previously frozen (independently of muscle). A significant higher a* values in

meat not frozen was also reported by Pietrasik and& Janz (2009) in *M. Semitendinous* of beef. Boles and Swan (2002a) reported also a decrease in redness after freezing of beef *M. Semimembranosus* from cattle, but the same effect was not observed for bull and steer (Boles & Swan, 2002b).

Yellowness (b*) of rhea meat was affected by muscles and storage time (Table 4.2). As observed for lightness and redness values, yellowness of rhea meat was higher in control samples than in samples submitted to frozen storage. In GN muscle values of b* were slightly lower than in IF muscle, but in both muscles yellowness decreases between 30 and 60 days and, thereafter, increases at 180 days of frozen storage. Muela et al. (2010) did not find differences in yellowness of lamb meat during frozen storage time, but b* decreased with de decrease in freezing rate.

Chroma indicates intensity of colour and is calculated as $\sqrt{(a_*^2 + b_*^2)}$. In rhea meat, Chroma was not influenced by muscle, but a high significant effect of storage time ($p \leq 0.0001$) was observed. Chroma values in control samples were higher than in samples stored frozen (independently of the duration of storage), demonstrating that rhea meat not frozen presents more intensity of colour than rhea meat previously frozen and thawed. During storage (from 30 to 180 days) chroma values varied slightly, but the differences were not significant.

Hue angle is derived from the arc tangent of b*/a*. Meat with larger values of hue angle is more yellow and discolored than meat presenting slight hue angles. Hue angle in rhea meat was influenced by muscle and storage time (Table 4.2). For both muscles the hue angle increased during storage, but in IF muscle the values were higher than in GN muscle. An increase in hue suggests a degree of change from red to yellow, probably due the oxidation of pigments (Mercier et al., 1998) during storage of meat, which in this study was more pronounced in *M. Iliofiburalis*. The occurrence of pigment oxidation of rhea meat during frozen storage was confirmed by analyzing the values for rhea meat discoloration (R630-R580) and rhea metmyoglobin accumulation (%MetMb). The decrease in R630-R580 for both rhea muscles during storage time indicates the decrease in oxymyoglobin content (Table 4.2). It can be due to a deficiency in enzyme activities and oxygenation process of myoglobin due to freezing/thawing processes, or to an increase in metmyoglobin during storage. Probably both reasons took part in this result, but effectively the percentage of metmyoblobin in rhea meat increased from ~30% in

control samples (independently of muscle) to 34 and 40% in GN and IF muscles, respectively, at the end of storage. The amount of metmyoglobin in rhea meat was highly influenced by muscle and storage time ($p \leq 0.0001$), but the greatest percentages of pigment oxidation were found in IF muscle, demonstrating a more susceptibility of this muscle to colour oxidation during storage in comparison to GN muscle. In a previously study, IF muscle of rhea also showed higher susceptibility to oxidation than GN muscle under air-packaged storage at 4 °C (Filgueras et al., 2010). One explanation was the fact that IF muscle presented more haem iron than GN muscle, which act as a potent pro-oxidant in meats.

4.4.3. Oxidative stability

Both muscle and storage time had highly significant effects on TBA-RS ($p \leq 0.0001$), with significant interaction between them (Table 4.3). TBA-RS increases during storage of rhea meat. Other authors have described a lipid oxidation increase at long frozen storage (Akköse & Aktas, 2008; Muela et al. 2010; Pereira Romanelli, Seriboni, & Barboza, 2006; Stika et al., 2007). The increase in lipid oxidations was more evident in IF than in GN muscle. At the end of storage (180 days) meat from IF muscle showed 2 times more MDA than GN muscle and the final values in IF muscle were high (5.4 mg MDA/kg meat). In rhea meat, Pereira et al. (2006) reported values between 0.46-0.82 mg MDA/kg meat in control samples and 1.80-2.55 after 60 days of storage of *M. Iliotibialis lateralis* at -18 °C. In lamb, Muela et al. (2010) reported an effect of freezing on TBA-RS, which high values of TBA-RS in thawed than in control samples.

Freezing affected meat oxidation probably because it causes damage in some cellular structures, especially membrane lipids (Monahan, Asghar, Gray, Euckley, & Morrisey 1994; Muela et al., 2010). Lipid oxidation is an autocatalyzed reaction in which some intermediate and final oxidation products have pro-oxidant effects. The oxidation of lipids and further free radical chain reactions could be aggravated by processing because processing procedures affect lipid in various ways. For example, heat treatment affects cellular structure, inactivates enzymes (including those with reducing activity), and releases oxygen from oxymyoglobin, creating conditions for hydrogen peroxide

production (Kanner, 1994). Cooking, especially at low temperatures for long times, has also the effect of releasing iron ions from haem groups (Novelli et al., 1998).

In this study, oxidation of lipids and proteins was determined in rhea muscles after cooking (100 °C, 30 min) in control, 30 d. and 180 d. samples. When cooking was included in general linear model, ANOVA revealed high significant effects of muscle and storage time ($p \leq 0.0001$) on TBA-RS values (Fig. 4.1; Table 4.4), with high significant interaction between both effects ($p \leq 0.0001$), but cooking did not affect TBA-RS ($p > 0.05$). A slight interaction between muscle and cooking treatment was found ($p \leq 0.05$). According to Gatellier, Santé-Lhoutellier, Portanguen, & Kondjoyan (2009b), TBA assay, which is the most common method used to measure lipid oxidation in food products, is not appropriate to quantify oxidation when meat is cooked at high temperatures. These authors observed a biphasic curve after different times and temperatures of heating of beef. The initial increasing phase correspond to the accumulation of aldehydes, such as malondialdehyde and hydroxyl-nonenal, the end products of polyunsaturated fatty acid oxidation in meat (Gray & Monahan, 1992; Melton, 1983; Sakai, Kuwazuru, Yamauchi, & Uchida, 1995), but other aldehydes as well as non-aldehydic compounds can also react with TBA (Gullien-Sans & Guzman-Chozas, 1998). The decreasing phase indicates an interaction between aldehydes and proteins, or a decomposition of such aldehydes to volatile compounds and thereby the decrease in the amount of aldehydes available to react with the thiobarbituric acid (Gatellier et al., 2009b).

The carbonyl groups are formed during protein oxidation by the reaction between NH or NH_2 side chain groups of amino acids and OH● (Berlett & Stadtman, 1997; Stadtman, 1990). The oxidation of basic amino acids, like lysine, histidine, and arginine, leads to an important decrease in nutritional value of meat, seeing that these amino acids (except arginine) are essential for humans. Phenylalanine and tryptophan are also essential amino acids for humans, while tyrosine is not required directly from the diet (except in diseased persons with phenylketonuria). Under the free radical attack aromatic amino acids, oxidation of aromatic amino acids can also negatively impact the nutritional value of meat. Carbonyl content was slightly affected by storage time ($p \leq 0.05$). The values ranged from 2.23 to 2.86 (Table 4.3), corresponding to GN muscle control and GN muscle stored for 90 days, respectively. Considering cooking in statistical model (Fig. 4.1; Table 4.4), carbonyl content was affected by storage time and cooking,

and significant effects were found between muscle and storage time, storage time and cooking, and muscle, storage time and cooking. The three aromatic amino acids phenylalanine, tyrosine, and tryptophan were affected by storage time (Table 4.3). Gatellier et al. (2009a) reported that cooking (60 °C, 30 min) had little effect on aromatic acid levels, while high effect on their stability was observed when higher temperatures were applied (100, 140 °C, 30 min). According to these authors, the stability of the three aromatic amino acids in bovine *M. Longissimus thoraci* during cooking decreased in the order tryptophan > phenylalanine > tyrosine. In the present study, storage time and cooking had a great effect on phenylalanine, tyrosine and tryptophan ($p \leq 0.0001$). After cooking, phenylalanine and tryptophan showed an increase in their levels when compared with fresh meat, but tyrosine levels decreased after cooking. This increase during storage and after cooking may be due to the losses observed during the thawing and cooking process, which increase the percentage meat concentration. In the case of tyrosine, the degradation process caused by cooking was more evident, and the level of this aromatic amino acid showed a decrease after cooking process.

4.5. CONCLUSION

Generally, no effect of muscle was observed on physical parameters, but muscle *Iliofiburalis* of rhea was more susceptible to oxidation than *Gastrocnemius pars interna*. It was more obvious concerning the percentage of metmyoglobin and TBA-RS values, which were higher in IF than in GN muscle. Variable modifications in quality attributes of rhea meat occurred during frozen storage time, which has been also reported in other kinds of meats. Prolonged frozen storage (up to 6 months) modified lightness, yellowness and discoloration of meat, but redness and chroma did not change, indicating that these attributes are more influenced by freezing/thawing than by storage duration. Lipid and protein oxidation of rhea meat was highly affected by cooking, but the extent of oxidation ranged according to muscle and duration of frozen storage applied to meat before heat treatment, i.e. cooking process interacted with muscle and storage time.

Long frozen storage affected rather slightly the overall quality of rhea meat. This storage mode could be favoured for exotic meat exportating countries.

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Table 4.1

Effect of muscle and frozen storage time on thawing, cooking and total losses of rhea meat (means ± SD).

Treatment	Attribute		
	Thawing loss (%)	**Cooking loss (%)**	**Total loss (%)**
M. Gastrocnemius pars interna			
Control	zero	42.0 ± 1.4^{a}	42.0 ± 1.4^{bc}
30 days	4.8 ± 3.0^{ab}	39.4 ± 1.8^{ab}	44.2 ± 2.7^{ab}
60 days	5.3 ± 2.9^{ab}	ND	-
90 days	6.7 ± 3.1^{ab}	ND	-
180 days	8.4 ± 2.7^{a}	38.4 ± 2.1^{b}	46.8 ± 3.7^{a}
M. Iliofiburalis			
Control	zero	38.4 ± 2.4^{b}	38.4 ± 2.4^{c}
30 days	2.9 ± 1.2^{b}	37.6 ± 2.5^{b}	40.1 ± 2.1^{bc}
60 days	4.9 ± 1.5^{ab}	ND	-
90 days	6.4 ± 2.6^{ab}	ND	-
180 days	7.0 ± 1.5^{a}	36.8 ± 2.4^{b}	43.8 ± 1.9^{ab}
P values			
Muscle effect	NS	**	***
Storage time effect	**	*	***
Muscle x Storage time	NS	NS	NS

NS: not significant;
ND: not determined;
^{abc} Means with the same superscript within same column do not differ significantly ($P \leq 0.05$);
* $p \leq 0.05$; ** $p \leq 0.001$; *** $p \leq 0.0001$.

Table 4.2

Effect of muscle and frozen storage time on colour parameters of rhea meat (means ± SD).

Treatment	Colour parameters						
	L*	**a***	**b***	**Chroma**	**Hue angle**	**R_{630}-R_{580}**	**%MetMb**
M. Gastrocnemius pars interna							
Control	36.2 ± 1.8^{a}	16.5 ± 0.8^{ab}	8.1 ± 0.7^{ab}	18.4 ± 0.8^{ab}	11.9 ± 1.8^{cde}	14.6 ± 1.2^{b}	29.2 ± 2.0^{e}
30 days	24.2 ± 3.7^{bcd}	13.7 ± 1.2^{bc}	6.9 ± 1.2^{bc}	15.4 ± 1.5^{bc}	12.7 ± 2.5^{cde}	6.5 ± 1.0^{c}	32.1 ± 0.7^{cde}
60 days	27.1 ± 4.2^{bc}	11.6 ± 1.7^{c}	5.1 ± 1.2^{c}	12.7 ± 2.0^{c}	9.79 ± 2.4^{e}	5.9 ± 0.8^{c}	32.2 ± 1.8^{cd}
90 days	25.2 ± 3.1^{bcd}	11.0 ± 1.3^{c}	5.1 ± 1.3^{c}	12.2 ± 1.7^{c}	10.4 ± 2.6^{de}	4.5 ± 0.9^{cd}	34.6 ± 1.5^{c}
180 days	22.0 ± 1.0^{cd}	13.1 ± 1.4^{c}	7.4 ± 1.1^{abc}	15.1 ± 1.7^{bc}	14.4 ± 2.1^{cde}	4.8 ± 0.8^{cd}	34.2 ± 1.2^{c}
M. Iliofiburalis							
Control	39.7 ± 3.6^{a}	17.2 ± 1.4^{a}	9.9 ± 1.5^{a}	19.9 ± 1.6^{a}	16.0 ± 4.0^{bcde}	16.9 ± 2.0^{a}	31.1 ± 0.8^{de}
30 days	24.9 ± 3.2^{bcd}	12.2 ± 3.2^{c}	7.7 ± 3.0^{abc}	14.5 ± 4.2^{c}	17.6 ± 4.7^{abc}	4.6 ± 1.7^{cd}	38.1 ± 2.3^{ab}
60 days	28.3 ± 3.3^{b}	11.5 ± 1.7^{c}	7.0 ± 2.1^{bc}	13.5 ± 2.4^{c}	17.0 ± 5.3^{bcd}	5.1 ± 1.2^{cd}	37.0 ± 2.4^{bc}
90 days	21.8 ± 2.9^{d}	11.6 ± 2.0^{c}	8.2 ± 1.6^{ab}	14.3 ± 2.1^{c}	22.1 ± 7.5^{ab}	3.3 ± 1.1^{d}	40.6 ± 2.7^{a}
180 days	23.5 ± 2.9^{bcd}	11.6 ± 2.5^{c}	8.8 ± 2.1^{ab}	14.6 ± 3.1^{bc}	24.1 ± 4.4^{a}	3.8 ± 1.5^{d}	39.8 ± 1.6^{ab}
P values							
Muscle effect	NS	NS	***	NS	***	NS	***
Storage time effect	***	***	***	***	**	***	***
Muscle x Storage time	*	NS	NS	NS	NS	**	*

NS: not significant;
^{abcde} Means with the same superscript within same column do not differ significantly ($P \leq 0.05$);
* $p \leq 0.05$; ** $p \leq 0.001$; *** $p \leq 0.0001$.

Table 4.3

Effect of muscle and frozen storage time on oxidative parameters of rhea meat (means ± SD).

Treatment			Aromatic amino acids (g/100g meat)		
	TBA-RS (mg MDA/kg tissue)	**Carbonyl (nmol/mg protein)**	**Phenylalanine**	**Tyrosine**	**Tryptophan**
M. Gastrocnemius pars interna					
Control	1.14 ± 0.40^{c}	2.23 ± 0.45^{c}	0.51 ± 0.21^{abc}	0.38 ± 0.09^{b}	0.15 ± 0.02^{b}
30 days	0.90 ± 0.33^{c}	2.59 ± 0.12^{abc}	0.47 ± 0.11^{bc}	0.52 ± 0.03^{a}	0.19 ± 0.02^{a}
60 days	1.03 ± 0.34^{c}	2.75 ± 0.22^{ab}	0.60 ± 0.15^{abc}	0.55 ± 0.05^{a}	0.18 ± 0.01^{a}
90 days	1.11 ± 0.40^{c}	2.86 ± 0.16^{a}	0.57 ± 0.13^{abc}	0.56 ± 0.03^{a}	0.19 ± 0.01^{a}
180 days	2.59 ± 0.71^{b}	2.32 ± 0.45^{bc}	0.71 ± 0.16^{ab}	0.57 ± 0.04^{a}	0.19 ± 0.01^{a}
M. Iliofiburalis					
Control	1.65 ± 0.25^{bc}	2.45 ± 0.31^{abc}	0.42 ± 0.12^{c}	0.38 ± 0.10^{b}	0.13 ± 0.03^{b}
30 days	1.80 ± 0.86^{bc}	2.50 ± 0.09^{abc}	0.45 ± 0.23^{bc}	0.55 ± 0.03^{a}	0.19 ± 0.01^{a}
60 days	2.82 ± 0.99^{b}	2.67 ± 0.17^{abc}	0.69 ± 0.19^{abc}	0.53 ± 0.06^{a}	0.19 ± 0.01^{a}
90 days	4.21 ± 1.16^{a}	2.54 ± 0.22^{abc}	0.69 ± 0.22^{abc}	0.50 ± 0.13^{a}	0.19 ± 0.02^{a}
180 days	5.40 ± 1.57^{a}	2.56 ± 0.51^{abc}	0.77 ± 0.13^{a}	0.54 ± 0.06^{a}	0.20 ± 0.01^{a}
P values					
Muscle effect	***	NS	NS	NS	NS
Storage time effect	***	*	***	***	***
Muscle x Storage time	***	NS	NS	NS	NS

NS: not significant;
^{abc} Means with the same superscript within same column do not differ significantly ($P \leq 0.05$);
* $p \leq 0.05$; ** $p \leq 0.001$; *** $p \leq 0.0001$.

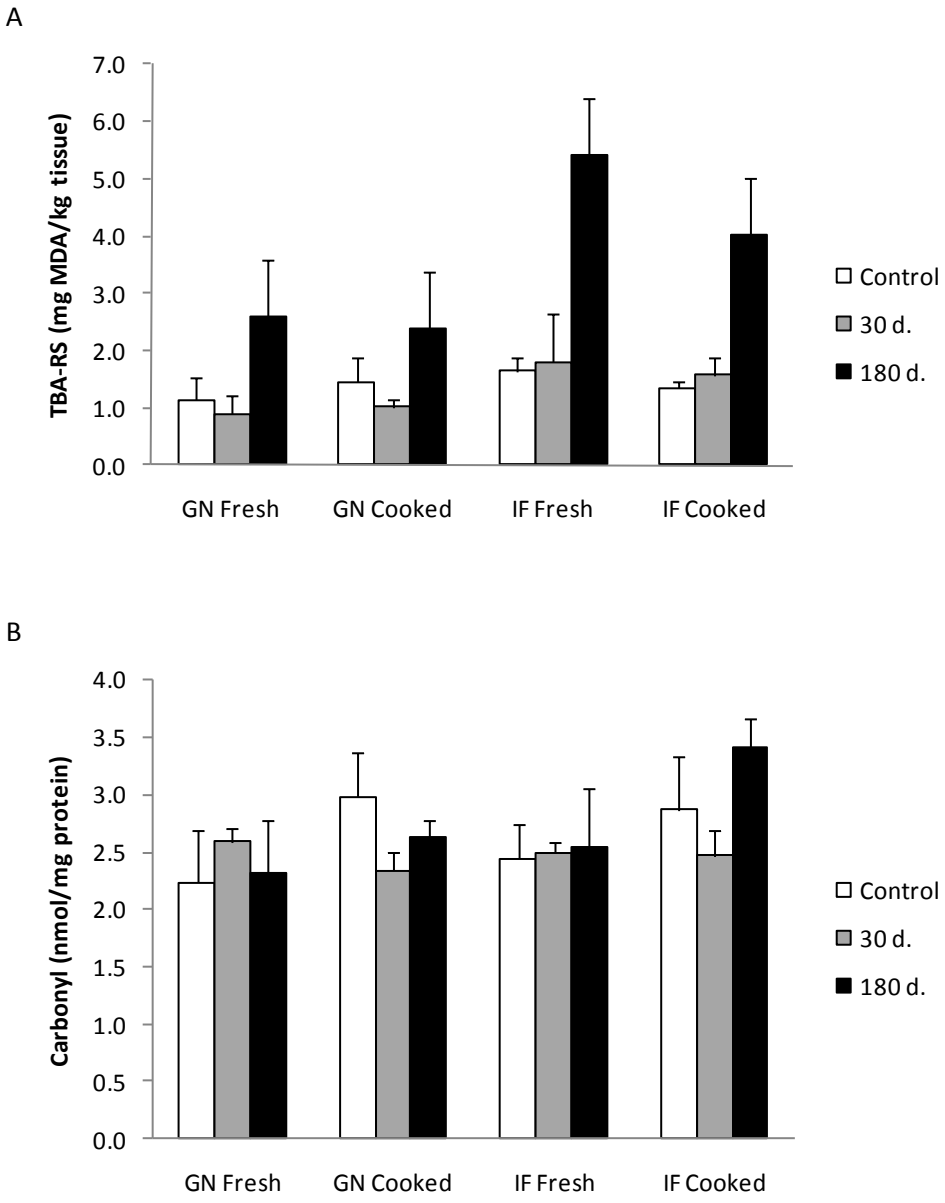

Figure 4.1. Level of TBA-RS (A) and carbonyl (B) in *M. Gastrocnemius pars interna* (GN) and *Iliofiburalis* (IF) of rhea, least squares means and standard deviations. Control: fresh samples at 24 h *post mortem*; 30 d.: samples stored for 30 days at -20 °C; 180 d.: samples stored for 180 days at -20 °C.

Table 4.4

Effect of muscle, frozen storage time, and cooking on oxidative parameters of rhea meat. Values are degree of freedom (df), means squares (ms), F value (F), and p value (p).

		TBA-RS	Carbonyl	Phenylalanine	Tyrosine	Tryptophane
Muscle						
	df	1	1	1	1	1
	ms	26.63	0.275	0.002	0.000	0.000
	F	51.39	2.854	0.067	0.084	0.296
	p	<.0001	NS	NS	NS	NS
Storage time						
	df	2	2	2	2	2
	ms	53.62	0.628	0.124	0.081	0.016
	F	103.4	6.726	4.979	15.66	27.54
	p	<.0001	0.002	0.009	<.0001	<.0001
Cooking						
	df	1	1	1	1	1
	ms	1.915	1.513	0.678	0.384	0.049
	F	3.696	17.15	27.13	74.27	82.18
	p	0.058	<.0001	<.0001	<.0001	<.0001
Muscle*Storage						
	df	2	2	2	2	2
	ms	8.874	1.079	0.013	0.000	0.000
	F	17.13	11.62	0.538	0.121	1.049
	P	<.0001	<.0001	NS	NS	NS
Muscle*Cooking						
	df	1	1	1	1	1
	ms	2.946	0.005	0.001	0.000	0.000
	F	5.686	0.058	0.058	0.138	0.017
	p	0.019	NS	NS	NS	NS
Storage *Cooking						
	df	2	2	2	2	2
	ms	1.573	1.078	0.311	0.081	0.005
	F	3.036	11.61	12.44	15.71	7.587
	p	NS	<.0001	<.0001	<.0001	0.001
Muscle* Storage *Cooking						
	df	2	2	2	2	2
	ms	0.343	1.053	0.008	0.003	0.001
	F	0.662	11.34	0.334	0.681	0.833
	p	NS	<.0001	NS	NS	NS

NS: not significant.

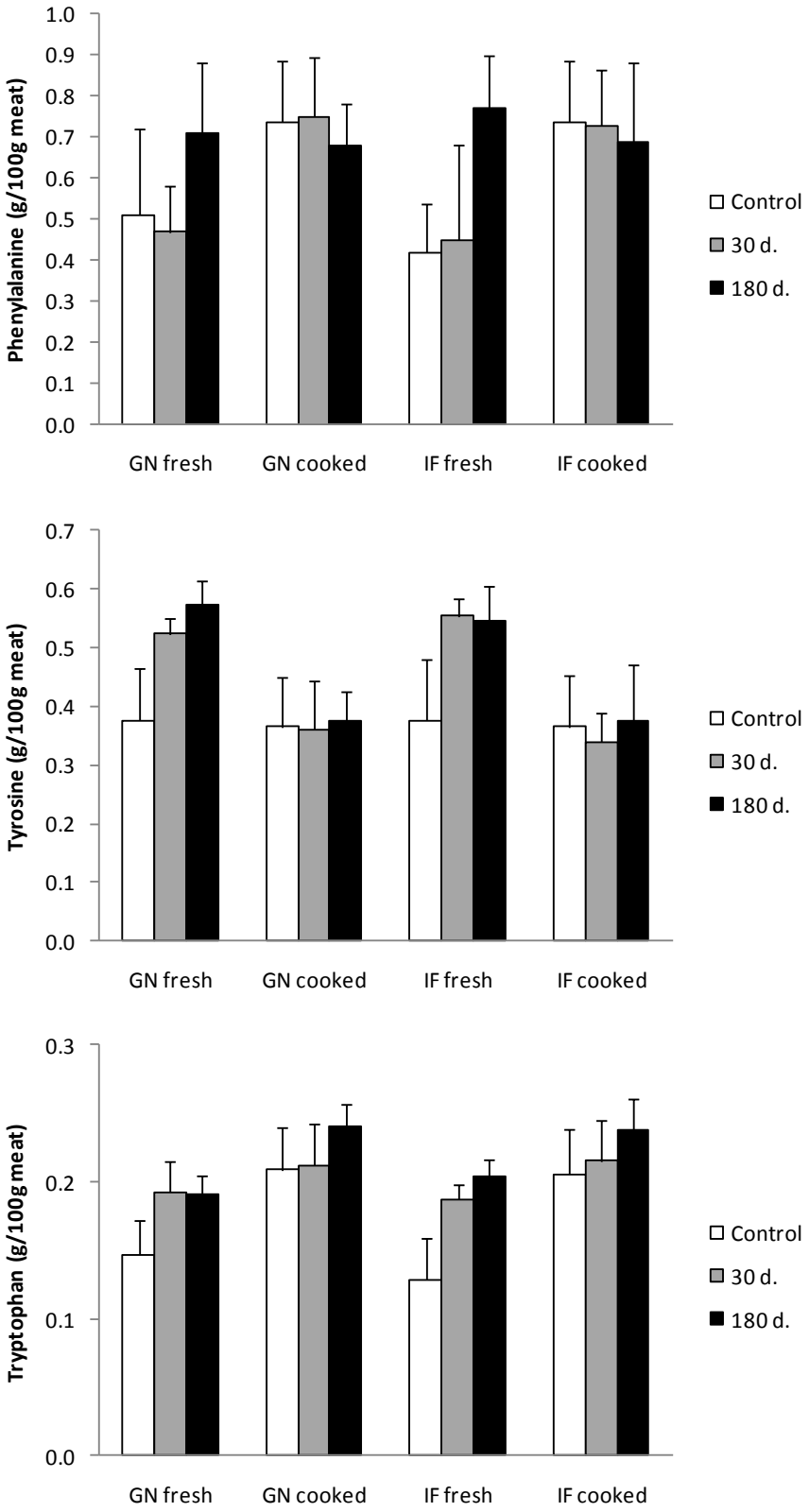

Figure 4.2. Aromatic amino acid levels in *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF) of rhea, least squares means and standard deviations. Control: fresh samples at 24 h *post mortem*; 30 d.: samples stored for 30 days at -20 °C; 180 d.: samples stored for 180 days at -20 °C.

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Chapter 5

Protein digestibility and nutritional value of rhea meat in association with storage and cooking processes

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(Original article submitted to Food Chemistry)

5.1. ABSTRACT

Nutritional value of proteins was investigated after storage and cooking of rhea *M. Gastrocnemius pars interna.* Oxidation of basic and aromatic amino acids, as well as surface hydrophobicity and aggregation state of proteins were determined in raw and cooked meat. In addition, myofibrillar proteins were exposed to proteases of the digestive tract (pepsin, trypsin and R-chymotrypsin) in conditions of pH and temperature that simulated stomach and duodenal digestion. Cooking had a great effect on protein hydrophobicity ($p < 0.0001$) and a small effect on tryptophan ($p < 0.05$), but did not affect phenylalanine and tyrosine contents. Storage influenced the fluorescence intensity in non-polar phase ($p < 0.0001$) indicating the presence of protein-aldehydes adducts after cooking. High content of Schiff bases were found in 5d samples demonstrating an implication of free amine group, probably from lysine. Cooking decreased the myofibrillar protein susceptibility to pepsin activity; however, the results for protease activity of trypsin and R-chymotrypsin were less obvious. Our findings support the importance of protein aggregation in the nutritional value of meat proteins.

Keywords: Rhea meat; protein oxidation; schiff bases; cooking; protein aggregates; rate of digestion.

5.2. INTRODUCTION

Meat is an important source of nutrients for human health and growth but it is frequently associated with a negative health image. Epidemiological studies have associated red meat consumption with the development of cardiovascular disease and colon cancer (Amanda, Michael, Mitchell, Albert, Arthur, & Rashmi, 2007; McAfee et al., 2010) due its high fat content and nutritionally inadequate fatty acid composition. Consumers have become more health conscious and have tended to consume low fat and low cholesterol foods. However, meat continues to be an important food group in the diet of many people and actually red meat forms part of the habitual balanced diet, particularly in developed countries. In this way, ratite meat such as rhea has been currently seen and marketed as a possible alternative to beef supply because it is a source of low fat red meat as well as an important source of proteins, minerals, and vitamins for human diet (Ramos, Cabrera, Del Puerto, & Saadoun, 2009; Sales, 1998; Sales et al., 1999). Nevertheless, studies on the nutritional quality of ratite's meats, mainly rhea, are particularly rare, which make difficult to evaluate the real benefits of these meat in comparison with other kinds of red meats.

Meat storage and cooking are necessary to achieve a safe and palatable product but these methods also play a significant role on the development of oxidation processes since they affect the physicochemical state of proteins and the bioavailability of their amino acids (Liu & Xiong, 2000a,b; Santé-Lhoutellier, Astruc, Marinova, Grève, & Gatellier, 2008; Tornberg, 2005). Oxidation of amino acid residues of protein generates a number of oxidation products, formation of protein-protein cross-linkages and fragmentation (Berlett & Stadtman, 1997; Davies, Designore, & Lin, 1987; Stadtman, 1990, 2002) and, in addition, Schiff bases can also be formed by the interaction between proteins and aldehydic products generated during lipid oxidation (Gatellier, Gomez, Gigaud, Berri, Bihan-Duval, & Santé-Lhoutellier, 2007; Renerre, Dumont, & Gatellier, 1996). Some authors have shown that aggregation of meat proteins was linked to protein hydrophobicity during cooking (Santé-Lhoutellier et al., 2008a) and it could influence the protein degradation by enzymes of the digestive tract (Kamin-Belsky, Brillon, Arav, & Shaklai, 1996; Liu et al., 2000a,b; Sante-Lhoutellier, Aubry, & Gatellier, 2007; Santé-Lhoutellier et al., 2008a), by reducing protein digestion rate and the

efficiency of amino acid assimilation. Consequently, the presence of non hydrolyzed proteins in digestive tract and their fermentation by colonic flora has been associated to an increase in the risk of colon cancer in humans (Evenepoel et al., 1998; Geypens et al., 1997).

Thus, the estimation of digestion rate of meat proteins can be of great interest in the evaluation of the nutritional and healthy qualities of meat products. Actually, a number of studies have been conducted on this subject in the last years (Blanquet et al., 2004, 2005; Gatellier & Santé-Lhoutellier, 2009; Kamin-Belsky et al., 1996; Liu & Xiong, 2000b; Sante-Lhoutellier et al., 2007). However, the connection between protein digestion rate and chemical oxidation of meat proteins has not already been completely clarified and results have frequently shown different outcomes. So, the present study aimed to evaluate the protein rate of digestion and nutritional quality of proteins from rhea muscle in association with meat processes (storage and cooking). Protein rate of digestion is presented and discussed in relationship with protein oxidation, denaturation and aggregation.

5.3. MATERIAL AND METHODS

5.3.1. Animals and samples

The experiment was carried out with rhea M. Gastrocnemius pars interna. Eight animals (Rhea americana) were slaughtered in the experimental slaughterhouse of INRA Theix Research Center, Theix, France. After 24 h of chilling, when the ultimate pH was reached, samples of muscle were cut in 2 cm thick steaks and stored in darkness at 4 °C for 5 days (5d) under air-packaged storage or 28 days (28d) under vacuum-packaged storage. Additional muscle samples were taken immediately after debone (1d), frozen in liquid nitrogen and stored at -80 °C for posterior chemical analyses.

5.3.2. Meat cooking

Samples in triplicates (5g) were sealed in polypropylene test tubes (inner diameter = 10 mm and thickness = 1 mm) and heated at 100 °C, in a digital temperature-

controlled dry bath (Block–heater, Stuart–Scientific) for 30 min. This treatment reflected meat cooking in an oven for which similar temperatures can be reached between meat core and meat surface. The centre temperature of the samples was measured with a digital thermometer fitted with a thin temperature probe. After cooking treatment, samples were cooled at room temperature for 15 min to reach 18-20 °C, and then frozen at -80 °C until its use.

5.3.3. Determination of cooking loss

Cooking loss (CL) was calculated as the ratio between the post-cooking and the pre-cooking weight, and expressed as a percentage (% CL = [(thawed weight - cooked weight)/thawed weight] x 100).

5.3.4. Determination of carbonyl content

To determine level of carbonyl group formation, muscle samples (1 g) were homogenized in 10 mL KCl 0.15 M + BHT 0.1 mM with a Polytron (1 min, medium speed) and proteins were treated with DNPH (2,4 dinitrophenylhydrazine) using the method of Oliver, Ahn, Moerman, Goldstein and Stadtman (1987). The carbonyl content was determined in fresh and cooked meat and the results were expressed as nanomoles of DNPH fixed per milligram of protein.

5.3.5. Determination of aromatic amino acids

The aromatic amino acids (AAA) were determined according to the method reported by Gatellier, Kondjoyan, Portanguen, Grève, Yoon and Santé-Lhoutellier (2009). The aromatic amino acids (phenylalanine, tyrosine and tryptophan) were determined in fresh and cooked samples. All measurements were performed in triplicate and the values were expressed as percentage of amino acid in fresh tissue (g/100 g meat).

5.3.6. Isolation of Myofibrils

Myofibrils were prepared according to the method of Ouali and Talmant (1990) with some modifications as outlined by Martinaud, Mercier, Marinova, Tassy, Gatellier and Renerre (1997).

5.3.7. Determination of Protein Surface Hydrophobicity

Hydrophobicity of myofibrillar proteins was determined using the hydrophobic chromophore BPB according to the method of Chelh, Gatellier and Santé-Lhoutellier (2007) with slight modifications. This method was based on the fixation of BPB with nonsolubilized myofibrillar proteins and the quantification of bound BPB. Myofibrillar proteins were suspended at the concentration of 2 mg/mL in 20 mM phosphate buffer at pH 6. To 1 mL of myofibril suspension was added and mixed well 40 µL of 1 mg/mL BPB (in distilled water). A control, without myofibrils, was done by the addition of 40 µL of 1 mg/mL BPB (in distilled water) to 1 mL of 20 mM phosphate buffer at pH 6. Samples and control were kept under agitation, at room temperature, during 10 min and then centrifuged 15 min at 4000g. The absorbance of supernatant, corresponding to free BPB, was measured at 595 nm against a blank of phosphate buffer. The amount of BPB bound in fresh and cooked meat, obtained by the difference between total and free BPB, was used in this study as an index of hydrophobicity. Each determination was performed in duplicate.

5.3.8. Determination of Fluorescent Pigments (Schiff bases)

Measurement of fluorescent pigments was performed in cooked rhea meat after 1d, 5d and 28d of storage, according to Renerre, Dumont and Gatellier (1996), modified by Gatellier, Santé-Lhoutellier, Portanguen and Kondjoyan (2009). Samples (0.5 g) were homogenized for 30 s with a polytron in 2.5 mL of phosphate 20 mM, NaCl 100 mM buffer solution at pH 6. One milliliter of this extract was diluted with 4 mL of solvent (dichloromethane: ethanol = 2:1 v/v ratio, with 0.1 mM of butylated hydroxytoluene added as antioxidant) and agitated for 15 min. After centrifugation (4000g for 15 min) two phases were observed; a polar upper phase composed of a mixture of water (70%) and ethanol (30%) and an non-polar lower phase composed of a mixture of

dichloromethane (80%) and ethanol (20%). These two phases were separated by an interface of insoluble material (probably composed of collagen and insoluble protein aggregates). Fluorescence intensities (FI) of the two phases were measured with a Perkin Elmer LS 50B spectrofluorometer in an excitation wavelength of 360 nm, excitation slit 10.0 nm, emission wavelength 390–600 nm, emission slit 10.0 nm and integration time 3 s. All measurements were performed in duplicate, at room temperature, and fluorescence values were expressed in arbitrary units (A.U.).

5.3.9. Determination of Aggregates by Rayleigh light scattering

The measurement of protein aggregates was performed using the method of Long, Zhang, Cheng and Bi (2008) adapted to myofibrils. Samples (0.5g) were homogenized (IKA T25 digital ULTRA-TURRAX) in 20 mL of a buffer 0.02 M Na2HPO4 at pH 6 and filtered on gauze to eliminate fibres of collagen. The amount of all protein concentration in the supernatant was estimated by Biuret method (Gornall, & Bardawill, 1949). Before measurement, myofibrillar protein concentration was adjusted to 0.04 mg in 1 mL of 20 mM phosphate buffer at pH 6. Then, 2 mL samples were placed into cuvettes under agitation and the measurement (4 technical repetitions) was performed with a synchronous scanning at λex = 300nm and a λem = 300nm (PERKIN ELMER, Luminescence Spectrometer LS 50 B). The results were expressed in arbitrary units (A.U.).

5.3.10. *In Vitro* Rate of Digestion

Protein rate of digestion by digestive tract enzymes was assessed as previously described by Santé-Lhoutellier et al. (2007) with slight modifications. Myofibrillar proteins were suspended in 33 mM glycine buffer at pH 1.8 (gastric pH), and final concentration was adjusted at 0.8 mg/mL. Proteins were first digested by gastric pepsin (5 units per mg of myofibrillar proteins) for 1 h at 37 °C. Digestion was stopped by the addition of 15% (final concentration) trichloroacetic acid (TCA) at various times (0, 10, 20, 30, 40, 60 min). After centrifugation for 10 min at 4000g, the content of hydrolyzed peptides in the soluble fraction was estimated by absorbance at 280 nm and the rate of proteolysis was expressed in optical density units by hour (ΔOD/h). The nonsoluble

fractions of the 30 min pepsin hydrolysate was washed twice in 33 mM glycine buffer at pH 8 (duodenal pH), and final concentration was adjusted at 0.8 mg/mL in this same buffer. Proteins were then hydrolyzed for 30 min at 37 °C by a mixture of trypsin and R-chymotrypsin (6.6 and 0.33 units/mg of myofibrillar proteins). Digestion was stopped by addition at various times (0, 5, 10, 20, 30 min) of 15% (final concentration) TCA, and the rate of proteolysis was determined as previously described. Each determination of digestibility by pepsin and pancreatic proteases was performed in duplicate in fresh and cooked rhea meat.

5.3.11. Statistical Analysis

A two ways analysis of variance (ANOVA) has been performed in order to test the level of statistical significance of time and cooking effects (and their interaction) on the variables. For significant effect, LS means were compared using Tukey test (SAS system). The relationships between the different parameters were assessed by calculation of Pearson correlation coefficients.

5.4. RESULTS AND DISCUSSION

5.4.1. Cooking loss

The results showed a significant difference between cooking loss at the different storage times: 1d, 5d and 28d (Table 1). The percentage of loss was slightly but significantly higher in meat stored for 1d (42%) than in meat stored for 5d under air-packaging or 28d under vacuum-packaging. This can be explained by the fact that an earlier drip loss generally occurs in samples during storage. The purge of part of free unbounded water of meat probably occurred before cooking (during storage), as observed in a previous study on rhea meat where the drip loss during storage of M. Gastrocnemius pars interna reached 1.90% after 5d of air-packaged storage and 4.91% after 28d of vacuum-packaged storage (Filgueras et al., 2010). Lower cooking loss was reported by Botha, Hoffman, & Britz (2007) in ostrich M. Gastrocnemius pars interna

muscle (36%), but the heat treatment applied for these authors (80 °C for 1 hour) was not the same applied in this study.

5.4.2. Carbonyl content

The carbonyl groups are formed during protein oxidation by the reaction between NH or NH2 side chain groups of amino acids and $OH^{\cdot}$ (Berlett & Stadtman, 1997; Stadtman, 1990, 2002). The oxidation of basic amino acids like lysine, histidine and arginine leads to an important decrease in nutritional value of meat, since lysine and histidine are essential amino acids for humans. In our experiment, carbonyl content in rhea fresh meat stored for 1d was 2.2 nmol of DNPH/mg of protein (Table 1), a value close to those reported by Martinaud et al. (1997) on beef, Morzel, Gatellier, Sayd, Renerre and Laville (2006) on pig myofibrils, Liu and Xiong (2000b) on chicken myosin and Santé-Lhoutellier, Engel and Gatellier (2008) on lamb. On rhea, in a recent work, Terevinto, Ramos, Castroman, Cabrera and Saadoun (2010) reported 2.27, 1.72 and 0.79 nmol of DNPH/mg protein in raw M. Iliotibialis lateralis, M. Obturatorius medialis and M. Iliofiburalis, respectively, immediately after animals' dead (day zero). Filgueras et al. (2010) have reported a significant increase in carbonyl content during the chilling of rhea meat for 5 days under air-packaging as the muscle was the Iliofiburalis, but not when the muscle used was Gastrocnemius pars interna. According to these authors, the chilling of Gastrocnemius pars interna for 5 days under air-packaging or for 28 days under vacuum-packaging did not significant increase the carbonyl content of fresh meat. The natural antioxidant protection of muscle varies according to the muscle, type and duration of storage, but it generally remains active for a few days after animal death (Renerre, Poncet, Mercier, Gatellier, & Metro, 1998). This can limit the carbonyl formation in the earlier period of storage of meat; however, the exposition of meat to the oxygen is known to increase oxidation processes. In our case, the lengthening of storage moderately affected the protein oxidation in M. Gastrocnemius pars interna because the chilling of meat under air-packaging storage was no longer than 5 days and meat stored for a long period (28d) was maintained under the absence of oxygen (vacuum-packaging).

The thermal treatment (100 °C, 30 min) of rhea meat induced to a significant increase in carbonyl content. With cooking, carbonyl content increased 33% in meat

stored for 1d and 39% in meat stored for 5d in cooked meat when compared with raw meat. In meat stored for 28d no significant increase in carbonyl content was observed after cooking (Table 1). Santé-Lhoutellier et al. (2008a) have also showed significant differences in carbonyl content after cooking of bovine myofibrillar proteins (100 °C, 30 min). The increase in protein oxidation after cooking can be attributed to the loss of antioxidant protection of muscles (Hoac, Daun, Trafikowska, Zackrisson, & Âkesson, 2006; Mey, Crum, & Decker, 1994); however, iron can also play an important role on oxidative processes since it can be released from heme and non-heme proteins (Garcia-Segovia, Andres-Bello, & Martinez-Monzo, 2007; Purchas, Busboom, & Wilkinson, 2006) in its catalytic form, promoting the formation of free radicals.

5.4.3. Aromatic amino acid contents

Determination of aromatic amino acids is of large relevance to assess the nutritional value of meat. Indeed, tryptophan and phenylalanine are essential amino acids for humans and have to be supplied through the diet. Storage time and cooking have no effects on the levels of the three aromatic amino acids (phenylalanine, tyrosine and tryptophan) in rhea meat. The results on the basis of meat weight are shown in Table 5.2. For phenylalanine and tyrosine contents, the duration of storage and cooking process did not affect the final contents in rhea meat, but tryptophan had its stability affected by cooking process ($p < 0.05$). In raw bovine M. Longissimus thoraci, Gatellier et al. (2009a) have reported similar values for phenylalanine (~ 0.6g/100g) and slight higher values for tyrosine (~ 0.6 g/100g) and tryptophan (0.2 mg/100g).

However, there were important cooking losses during the heat of rhea meat (of about 40% of losses; Table 5.1). Thus, on the basis of meat weight (mg/100g meat) the tryptophan contents increased after cooking, but calculating the content of aromatic amino acids on the basis of protein level (g/100 of protein) it was observed a decrease in the levels of tryptophan and also in the level of the phenylalanine and tyrosine after heat. For example, based on protein level the rhea meat stored for 28d presented a decrease of 39% in phenylalanine, 38% in tyrosine and 22% tryptophan levels after cooking. Considering the values on the basis of protein levels, Gatellier et al. (2009a) have also observed a decrease in aromatic amino acid contents after cooking of beef. On

the basis of protein level these authors reported a decrease of 38% in phenylalanine, 69% in tyrosine and 32% in tryptophan content.

5.4.4. Surface hydrophobicity of proteins (BPB bounds)

Hydrophobicity can be a suitable parameter to estimate protein denaturation because it monitors subtle changes in the chemical and physical states of protein. The increase in protein surface hydrophobicity indicates the unfolding of myofibrillar proteins and exposure of non-polar amino acids to their surface (Chelh, Gatellier, & Santé-Lhoutellier, 2006; Van der Plancken, Van Loey, & Hendrickx, 2006). The ANOVA revealed a cooking effect on the hydrophobicity of myofibrillar proteins of rhea M. Gastrocnemius pars interna. An important (approximately 3 times) and significant ($p < 0.0001$) increase in BPB bounds was observed after thermal treatment (Table 1) for all storage times applied in this study. Our results are similar to those reported by Chelh et al. (2006) who have found ~60 μg of BPB bounds in pig myofibrils after heat treatment (70 °C, 60 min). In beef, Santé-Lhoutellier et al. (2008a) also found a great increase in BPB bounds after heat treatment (100 °C, 30 min), but their values were always inferior than 40 μg.

Myofibrillar proteins are important structural proteins implicated in tenderness and water holding capacity of meat and information related to their denaturation pattern is of great importance in meat technology. However, changes in chemical and physical state of proteins can also be implicated in the decrease of proteolytic susceptibility of skeletal muscle myofibrillar proteins and, consequently, to reduce protein digestibility of meat (Morzel et al., 2006).

5.4.5. Fluorescent pigments and aggregates

According to Dean, Stocker and Davies (1997), Schiff bases are short-lived species formed by the reaction of carbonyl groups with amines, and can be formed during exposure of proteins to lipid-derivate aldehydes, autoxidizing sugars, and amino acid-derived aldehydes. Schiff bases formed are unstable and often are rapidly followed by Amadori rearrangements. Table 5.3 presents the data of fluorescent pigments in the polar (water/ethanol) and non-polar (dichloromethane/ethanol) phases. The

distribution of fluorescent pigments in the two phases can be related to differences in pigment composition and solubility. In our study it was observed a peak with a maximum emission at 430 nm in non-polar phase and two peaks in the upper polar phase with a maximum emission at 410 nm and 520 nm. The ANOVA revealed an effect of storage time ($p < 0.0001$) on the content of fluorescent pigment in non-polar phase (Table 3). Meat stored for 5d under air-packaging showed 1.5 times more fluorescence in non-polar phase than meat stored for 1d; and 0.6 times more fluorescence in non-polar phase than meat stored for 28d under vacuum-packaging.

In food, during the heating process, two pathways of fluorescent pigment formation have been identified. The first is mainly oxidative and implicates the reaction of proteins with the aldehydic products of lipid peroxidation. The second implicates the reaction of proteins with reducing sugars. The distribution of fluorescent pigments is based on the difference of solubility of protein-sugar adducts and protein-aldehydes adducts. So, according to Gatellier et al. (2009b), proteins with high amounts of linked aldehydes are soluble pigments and concentrate preferentially in the non-polar phase. In addition to differences in solubility, the polarity of the proteins can also influence the fluorescent pigment distribution. For example, heating increases the protein surface hydrophobicity of meat proteins (Chelh et al., 2006) and egg proteins (Van der Plancken et al., 2006) and consequently can enhance their solubility in the non-polar medium. In this way, we have observed higher fluorescence in the non-polar phase of cooked meat stored for 5d under air-packaging than in meat stored for 28d under vacuum. This could be explained by the lipid oxidation and production of aldehydes during storage under oxygen permeable film (Filgueras et al., 2010) which interact with proteins forming soluble fluorescent pigments that accumulate in the non-polar phase. Filgueras et al. (2010) have shown that, under similar conditions than used in the present study, lipid oxidation of M. Gastrocnemius pars interna of rhea increased 60% after 5d of storage when compared with meat immediately after debone (24 h post mortem).

The important fluorescence wavelength shift observed in the two phases indicated that pigments with different chemical structures or environments were produced during the heating process, but the precise chemical structure of these compounds was not investigated in this study. Spectra with two emission peaks have already been described when proteins were exposed to aldehydes or sugars, but

attribution of each peak to a specific compound still unclear. In a study that investigated the interaction between glucose-6-phosphate dehydrogenase and 4-hydroxy-2-nonenal (an important aldehyde of the lipid peroxidation), Friguet, Stadtman and Szweda (1994) described a fluorescence spectrum with maximum emission at 415 nm and a distinct shoulder between 450 and 550 nm. It was attributed to cross-linked proteins. However, the temperature used in this referenced study was not specified which makes difficult comparison with the present work. In milk, Kulmyrzaev and Dufour (2002) have also observed two peaks of fluorescence after heating which appeared at the same wavelengths as those observed in this study. These authors attributed the first peak to Maillard fluorescent products and the second peak to FADH, a co-factor of enzymes found in milk. Similarly, two such fluorescent peaks were reported in UHT (ultra high temperature) milk by Schamberger and Labuza (2007) who have also attributed the fluorescence to Maillard products.

For study the aggregate formation in rhea meat a light scattering method (Rayleigh light scattering) was applied. The level of protein aggregates in fresh and cooked meat of rhea is shown in Fig. 5.1. The light scattering was highly affected by cooking, demonstrating a higher content of aggregates in cooked meat than in raw meat of rhea. One explanation for the increase in aggregates in meat after cooking is that thermal treatment favors exposition of thiol group and interior hydrophobic residues which lead to the formation of disulfide bridges, hydrogen bonds and hydrophobic interactions between proteins and other compounds (Su, He, & Qi, 2008). Such reactions and interactions promote protein aggregation via non-native and expanded conformational state of proteins.

5.4.6. In vitro digestion rate of myofibrillar proteins

The ANOVA test revealed a highly significant effect of cooking ($p < 0.0001$) but not of storage duration ($p > 0.05$) on pepsin activity. The results for pepsin activity in rhea myofibrillar proteins after heat (100 °C, 30 min) are shown in Fig. 5.2. Our results are in accordance with the results reported by Santé-Lhoutellier et al. (2008a) that have also observed a decrease of pepsin activity after beef cooking. In fact, process giving high level of protein oxidation (as cooking) generally affects protein digestion rate because it lead to cleavages, loss of accessibility of protease to protein and modification of the

cutting site of amino acids. According to Berlett and Stadtman (1997), the oxidative cleavage of proteins can difficult protein hydrolyze due the formation of peptides in which the N-terminal amino acid is blocked by α-ketoacyl derivates.

Moreover, the aromatic amino acid residues are generally hydrolyzed by pepsin in stomach and are among the preferred targets for reactive oxygen species (ROS) attack: tryptophan residues are readily oxidized to formylkynurenine and kynurenine, as well as to various hydroxyl derivates, and phenylalanine and tyrosine residues also yield a number of hydroxyl derivates (Berlett & Stadtman, 1997; Dean et al. 1997; Stadtman, 2003). In addition, carbonyl groups can also be introduced into proteins by reaction with aldehydes produced during lipid peroxidation (Berlett & Stadtman, 1997; Estenbauer, Schaur & Zollner, 1991; Uchida & Stadtman, 1993) or by reaction with other reactive carbonyl derivates (as ketoamines, ketoaldehydes, deoxyosones) generated as a consequence of the reaction of reducing sugars or their oxidation products with lysine residues of proteins (glycation and glycosidation reactions) (Baynes, 1991; Berlett & Stadtman, 1997).

As a consequence, such oxidized forms of proteins and proteins modified by glycation or lipid peroxidation products seem to be resistant to proteolysis and can inhibit the ability of proteases to degrade proteins (Friguet et al., 1994; Grune, Reinheckel, Joshi, & Davies, 1995). However, this information is controversial. According to Dean et al. (1997), biphasic effects have also been reported, i.e. while limited oxidation can enhance susceptibility of proteins to protease, more extensive oxidation may also be associated with the increase of resistance to proteases.

The rate of digestion by pancreatic proteases (Trypsin + R-chymotrypsin) demonstrated that cooking did not affect the digestibility of myofibrillar proteins of rhea meat ($p < 0.001$) (Fig. 3). What is more, the heating treatment (100 °C, 30 min) even induced to a significant increase in pancreatic protease activity 5d samples ($p < 0.05$). This increase is curious and difficult to explain.

It is known that proteins are generally denaturated and oxidized with heating treatment, but the extent of transformations depends on the duration and the intensity of heating. Oxidation of different amino acids generates different protein changes (Berlett & Stadtman, 1997). The two pancreatic enzymes (trypsin and R-chymotrypsin) have different cutting sites (i.e. arginine and lysine are cut by trypsin and aromatic

amino acids and methionine are cut by chymotrypsin) (Nelson & Cox, 2005). So, while the recognition of the cutting site of proteins by the trypsin could be negatively influenced by the oxidation of basic amino acids and presence of carbonyl groups, the protein conformational changes provoked by heating (increase in protein hydrophobicity) and the oxidation of aromatic amino acids could enhance chymotrypsin activity.

As related by Liu and Xiong (2000b), some essential amino acid residues (lysine, methionine, phenylalanine and tryptophan) are more susceptible to free radical attack and then meat proteins exposed to strong oxidative processes (as high temperature conditions) would conceivably have a decrease in their nutritional quality due to the loss of these amino acids. In this way, Santé-Lhoutellier et al. (2007, 2008a) have demonstrated that under severe conditions as in a strong hydroxyl radical generating system, which enhance myosin molecules to cross-link, the oxidized protein have seemed more resistant to proteolysis by digestive enzymes (Santé-Lhoutellier et al., 2007, 2008a).

Therefore, in view of its capacity to monitor subtle changes in the chemical and physical state of protein, hydrophobicity can be a suitable parameter to estimate protein denaturation (unfolding of proteins). In order to localize this change in protein structure at the cellular level, Santé-Lhoutellier et al. (2008a) used microscopic observations of muscle tissue stained by the BPB after 5 and 45 minutes of cooking at 100 °C. An important increase in of blue coloration was observed after cooking, which was almost the same after 5 or 45 min. This result confirms that changes in protein structure appear very early during meat cooking. After unfolding, the next step in the structural changes to occur on heating is protein-protein interaction, resulting in aggregation.

Microscopy observations for detection and localization of oxidized proteins in muscle cells by Astruc, Marinova, Labas, Gatellier and Santé-Lhoutellier (2007) showed an important increase of both myofibrillar and membrane oxidation with cooking time. Myofibrillar protein denaturation and aggregation induced by heating, leads to heterogeneity of the oxidized protein distribution and increase the extracellular space and an important disintegration of collagen fibers.

5.5. CONCLUSION

The present study showed that storage time/aging had less impact than cooking on proteins state. The protein aggregation resulted both from hydrophobic and covalent interactions. Moreover, it was observed a combined effect of storage under air and heating on protein aggregation The nutritional value of proteins was affected as demonstrated by the decrease of pepsine activity rate, but for trypsin chymotrypsin the results remained difficult to explain. These findings warrant further study into the characterization of the aggregates to be able, in the future to adapt process to optimal nutritional value of proteins.

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Table 5.1

Effect of refrigerated storage time and cooking (100 °C, 30 min) on physicochemical parameters of rhea *M. Gastrocnemius pars interna* meat (means ± SEM).

	Cooking loss *(%)*	**Carbonyl** *(nmol/mg protein)*	**BPB bounds** *(µg)*
Fresh Samples			
1day post mortem	-	2.2 ± 0.4^{b}	61.9 ± 12.7^{b}
*5 days post mortem**	-	2.3 ± 0.4^{b}	63.8 ± 13.8^{b}
*28 days post mortem***	-	2.8 ± 0.5^{ab}	73.5 ± 17.9^{b}
Cooked samples			
1day post mortem	41.9 ± 1.4^{a}	3.0 ± 0.4^{a}	192.5 ± 4.5^{a}
*5 days post mortem**	40.5 ± 1.1^{ab}	3.2 ± 0.4^{a}	193.9 ± 4.9^{a}
*28 days post mortem***	40.0 ± 1.0^{b}	2.7 ± 0.3^{ab}	191.3 ± 6.3^{a}
P values			
Storage time effect	0.009	NS	NS
Cooking effect	-	<.0001	<.0001
Time x Cooking	-	0.0032	NS

NS: not significant;
[ab] Means with the same superscript within same column do not differ significantly ($P \leq 0.05$);
*Samples conserved under air-packaging during storage;
**Samples conserved under vacuum-packaging during storage.

Table 5.2

Effect of refrigerated storage time and cooking (100 °C, 30 min) on aromatic amino acid contents of rhea *M. Gastrocnemius pars interna* meat (means ± SEM).

	Aromatic amino acids (g/100g meat)		
Fresh samples	**Phenylalanine**	**Tyrosine**	**Tryptophan**
1day post mortem	0.51 ± 0.20	0.38 ± 0.09	0.14 ± 0.02[b]
*5 days post mortem**	0.57 ± 0.09	0.43 ± 0.04	0.15 ± 0.01[ab]
*28 days post mortem***	0.55 ± 0.13	0.43 ± 0.04	0.15 ± 0.02[ab]
Cooked samples			
1day post mortem	0.69 ± 0.24	0.37 ± 0.08	0.20 ± 0.03[a]
*5 days post mortem**	0.58 ± 0.25	0.31 ± 0.15	0.19 ± 0.08[ab]
*28 days post mortem***	0.46 ± 0.22	0.37 ± 0.05	0.16 ± 0.01[ab]
P values			
Storage time effect	NS	NS	NS
Cooking effect	NS	NS	0.046
Time x Cooking	NS	NS	NS

NS: not significant;
[ab] Means with the same superscript within same column do not differ significantly ($P \leq 0.05$);
*Samples conserved under air-packaging during storage;
**Samples conserved under vacuum-packaging during storage.

Table 5.3

Effect of previously storage time on fluorescent pigments (Schiff bases) of rhea *M. Gastrocnemius pars interna* after cooking.

	Fluorescence intensity (a.u.)		
	Non-polar phase	**Polar phase (peak 1)**	**Polar phase (peak 2)**
Cooked samples			
1day post mortem	12.31 ± 1.24 [c]	27.55 ± 1.01	43.05 ± 12.51
*5 days post mortem**	30.82 ± 9.87 [a]	29.74 ± 3.70	37.29 ± 2.54
*28 days post mortem***	19.66 ± 5.14 [b]	32.28 ± 7.28	46.02 ± 12.13
P values			
Storage time effect	<.0001	NS	NS

NS: not significant;
[ab] Means with the same superscript within same column do not differ significantly ($P \leq 0.05$);
*Samples conserved under air-packaged storage;
**Samples conserved under vacuum-packaged storage.

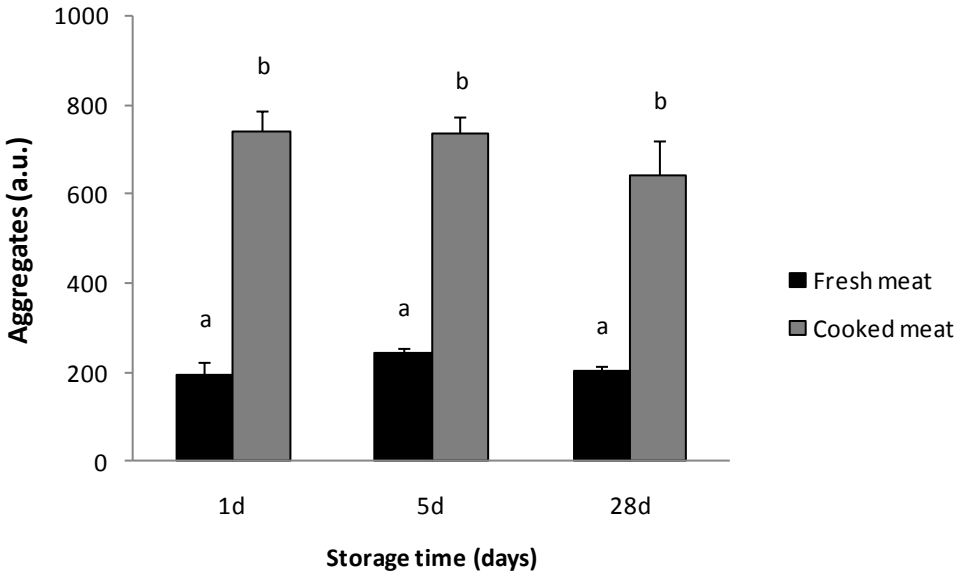

Figure 5.1. Effect of refrigerated storage time and cooking (100 °C, 30 min) on myofibrillar protein aggregation of rhea *M. Gastrocnemius pars interna*, measured by Rayleigh light scattering (means ± SEM). Values not bearing common letters differ significantly ($p \leq 0.05$). 1d: debone day, 24h *post mortem*; 5d: 5 days *post mortem*; 28d = 28 days *post mortem*.

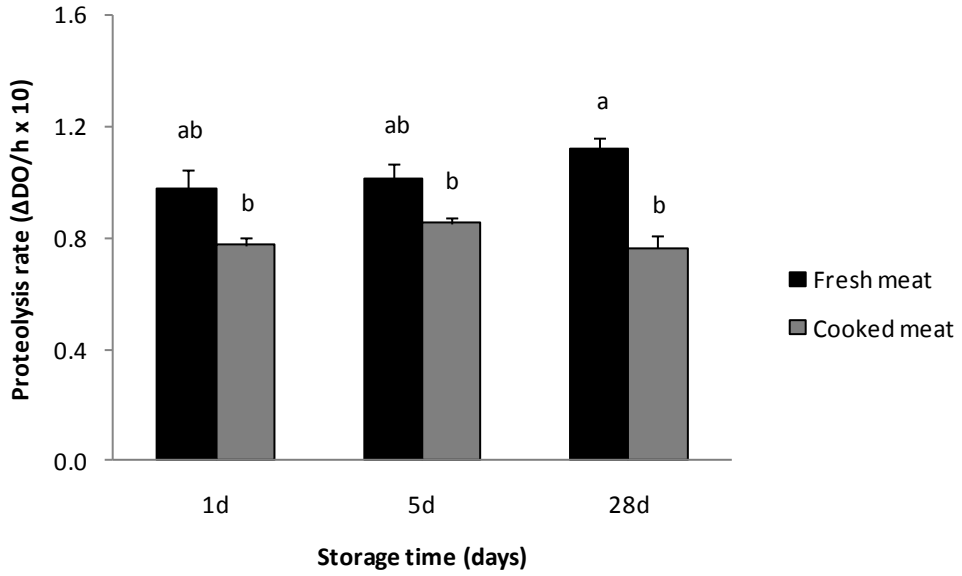

Figure 5.2. Effect of refrigerated storage time and cooking (100 °C, 30 min) on proteolysis rate of rhea *M. Gastrocnemius pars interna* myofibrillar proteins by gastric pepsin (means ± SEM). Values not bearing common letters differ significantly ($p < 0.05$). 1d: debone day, 24h *post mortem*; 5d: 5 days *post mortem*; 28d = 28 days *post mortem*.

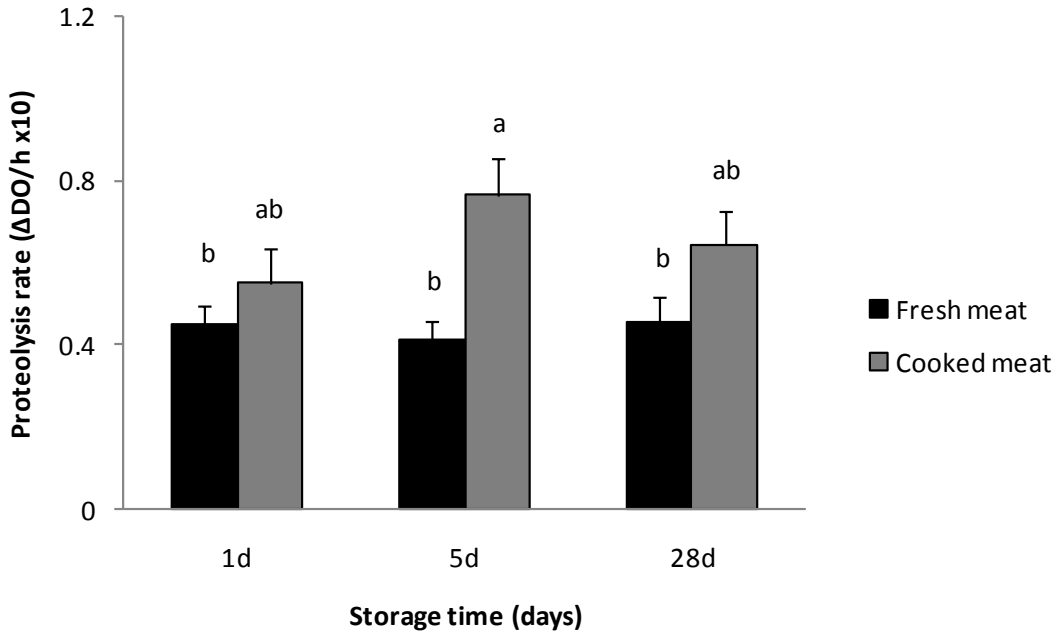

Figure 5.3. Effect of storage time and cooking (100 °C, 30 min) on proteolysis rate of rhea *M. Gastrocnemius pars interna* myofibrillar proteins, previously treated with pepsin, by pancreatic trypsin + α-chymotrypsin (means ± SEM). Values not bearing common letters differ significantly ($p < 0.05$). 1d: debone day, 24h *post mortem*; 5d: 5 days *post mortem*; 28d = 28 days *post mortem*.

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GENERAL CONCLUSION

Most of the ratites are found in South Africa, Australia and Brazil. The valorisation of exotic animals and their meat presents an indeniable interest for Brazil, besides the feathers and the leather. Brazil continues to strengthen its position as the world's leading meat exporter, with shipments of beef, poultry and pork together generating more than $10 billion last year. With some of the lowest production costs in the world, the Brazilian livestock sector is able to supply an increasingly sophisticated range of products at prices which few of its competitors are able to match. Meat exportations are often achieved with frozen meat. This technology extends the shelf life of the products by stabilizing the microbial spoliage but many biochemical reactions still occur during the storage affecting the sensorial and nutritional quality of meat. In the case of rhea muscles, little information on meat quality was available. Therefore the design of the experiment was elaborated with rheas from an experimental farm in Provins (France) slaughtered in controlled conditions at INRA facilities. Two distinct muscles (*M. Gastrocnemius pars interna* and *M. Iliofiburalis*) were studied in different conditions of storage to get deeper knowledges in the determinism of the nutritional quality of rhea meat and in meat in general after processing. The study was conducted 1) to characterise the conversion of muscle into meat in *rhea americana* and to evaluate the oxidative stability of *M. Gastrocnemius pars interna* and *M. Iliofiburalis* during chilling and frozen storage. ; 2) to assess the protein rate of digestion and nutritional quality of rhea meat proteins in relation with oxidation, denaturation, and aggregation; and 3) to establish links between the physicochemical changes post mortem and the histochemical/ultrastructural characteristics of both rhea muscles.

This study has provided new knowledge on the physicochemical, biochemical, sensorial, nutritional, histochemical and structural characteristics of rhea meat. Most of the available studies on ratite's meat were based on ostrich meat production and chemical composition of ostrich meat and did not link the characteristics of muscles

with meat quality. Very few studies focused on quality of rhea meat. So, in this project, an intense investigation on oxidative stability during storage (chilling and frozen), influence of meat processes (ageing and cooking) in nutritional quality and metabolic and contractile characteristic of muscles was conducted, and the summary of the results are described below.

Differently from other studies that have shown an intermediate to high ultimate pH in ratite's muscles (Berge et al. 1997; Sales, 1996; Sales & Mellett, 1996; Seydim et al. 2006), the ultimate pH of *M. Gastrocnemius pars interna* (GN) and *M. Iliofiburalis* (IF) of rhea was normal, comprised between 5.4 and 5.6. In addition, after 24 h post mortem it was observed a high concentration of glycogen in rhea muscles, mainly in IF muscle, which presented a residual glycogen concentration of 42 μmol/g. Total lipids and cholesterol contents were also higher in IF than in GN muscle, and the fatty acids predominant in both muscle were linolenic (C18:2 n-6), oleic (C18:1) and palmitic fatty acid (C18:0). The superoxide dismutase (SOD) activity did not differ between rhea muscles, but catalase was significantly higher in GN muscle. The rhea IF and GN muscles differed in their amount of haeminic iron, with 30% more haeminic iron in IF muscle. Rhea meat presented higher content of myoglobin in rhea meat than in chicken, beef, lamb and pork (Lombardi-Boccia et al. 2002), assessed through the quantification of haeminic iron.

In relation to oxidative stability of rhea muscles during storage this study showed that chilling under air-packaging was not appropriated to preserve colour, lipid and protein quality of rhea meat, mainly in meat from IF muscle. This muscle was highly susceptible to oxidative processes when exposed to oxygen. After 3 days under air-packaged storage IF muscle presented significantly higher percentage of metmyoglobin accumulation, lipid oxidation and protein oxidation than GN muscle. The myoglobin accumulation reached 47% at 5 days of storage in IF muscle against 33% in GN muscle. In sensorial analysis, at 3 days of storage, meat from IF muscle was rejected by 1 consumer out of 2, while GN was considered red in colour and having good appearance.

On the other hand, chilling under vacuum-packaging was totally efficient to contain oxidation and should be prioritized for industry instead of air-packaged storage. The colour of both muscles was stable and no increase in TBA-RS and carbonyl content was observed during the four weeks. However, the same was not confirmed in frozen

storage of rhea meat. Even under vacuum-packaging and low temperature (-20 °C), once again IF muscle demonstrated higher oxidation instability, with significant increase in metmyoglobin and TBA-RS after 90 days of frozen storage. This result is quiet important for meat exports and deserves further investigations, especially by reninforcing the antioxydant defense in the muscles.

The higher instability of IF muscle to oxidation process could be partially explained by the higher content of haeminic iron and higher lipid content found in such muscle when compared to GN muscle. However, the extent of oxidation in IF muscle also indicates a possible implication of residual glycogen and, consequently, a possible influence of muscle fibre metabolism in the development of myoglobin, lipid and protein oxidation processes of rhea. Pearson's correlation between residual glycogen and percentage of myoglobin oxidation ($r = 0.86$; $p < 0.0001$), residual glycogen and TBA-RS ($r = 0.81$; $p < 0.001$), and residual glycogen and carbonyls content ($r = 0.72$; $p < 0.01$) in air-packaging chilled meat were positive and significant.

In this way, trying to understand such comportment of rhea muscles face the oxidation processes, the structure and ultrastructure characteristics of rhea muscles was analysed. It confirmed high glycogen concentration in rhea muscles (higher in IF than in GN), but it also demonstrated an oxidative capacity of fibres. The histochemistry indicated the presence absolute of one type of fibres in both muscles of rhea, i.e. all muscles fibres of IF and GN muscles analyzed were classified as Fast-twitch Oxidative Glycolytic (FOG) type, according to Peter et al. (1972). This results indicates that mitochondrial oxidative capacity of rhea muscles and the high concentration of residual glycogen, mainly in IF muscle, may be involved in oxidative instability of meat exposed to oxygen. Higher proportion of oxidative fibres coincides with higher concentration of mitochondria in muscles and differences in oxygen consumption rate in muscles was already demonstrated to be involved to myoglobin autoxidation rate (Renerre & Labas, 1987). Interestingly the distribution of mitochondria differed between the 2 muscles, where IF showed mitochondria in between the myofibers. In general mitochondria are localized closed the cell membrane.

To finish, the study of nutritional value of rhea meat proteins demonstrated that thermal treatment greatly affected protein hydrophobicity, which indicates the protein conformational changes and denaturation. Tyrosine stability ($p < 0.0001$) was also

prejudiced by cooking, but phenylalanine and tryptophan contents remained stable. The duration and condition of meat storage (air-packaging or vacuum packaging) before cooking affected the fluorescence intensity in non-polar phase ($p < 0.0001$), which is associated to the cross-linking between proteins and aldehydes produced by liporeroxidation (Gatellier et al., 2009). Higher content of Schiff bases were found in samples previously stored for 5d under air-packaging, demonstrating the implication of free amine group of proteins, probably from lysine, in aggregation. Meat cooking decreased the myofibrillar protein susceptibility to pepsin, but for protease activity of trypsin and R-chymotrypsin, the results were variable and less obvious. The classic criteria for evaluating the quality of a protein source based on amino acids composition require including the capacity of dietary proteins to release, during the digestion, peptides having a local or systemic biological impact and to take the rate of digestion which can be modulated by processing conditions.

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APPENDIX A:

Communication article in *VIII Journées de la Recherche Avicole*

IMPACT DU MODE DE CONSERVATION SUR LA COULEUR ET L'ACCEPTABILITE DE LA VIANDE DE NANDOU (RHEA AMERICANA)

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RÉSUMÉ

Au cours des dernières années, la production de viande de ratites (autruches, émeus et nandous) a augmenté. La viande de nandou est maigre et rouge avec un goût proche de celle du bœuf. Actuellement, les seules études menées sur la qualité de la viande de nandou concerne l'aspect sanitaire et la composition. L'objectif de cette étude est d'évaluer l'impact du mode de conditionnement sur la stabilité de la couleur et l'évolution des processus oxydatifs de la viande de nandou en fonction du muscle. Huit nandous âgés de 15 mois en moyenne on été abattus et les carcasses réfrigérées avant d'être découpées à 24h *post mortem*. Les muscles *Gastrocnemius pars interna* (GN) et *Iliofiburalis* (IF) ont été découpés et conservés : a) à 4°C sous film perméable à l'oxygène pendant 5 jours ; b) à 4°C sous vide pendant 28 jours. Un suivi des paramètres de couleur (système CIELab*), de l'oxydation lipidique (SR-TBA) et protéique (carbonyles) ont été réalisés. La teneur en fer héminique a été déterminée à J1 pour tous les échantillons. Pour les échantillons conservés sous film perméable à l'oxygène, un jury a noté l'aspect de la tranche et indiqué sa décision d'achat. Le muscle GN conservé sous film perméable à l'oxygène se caractérise par une grande stabilité de la couleur, moins d'oxydation lipidique et protéique. A l'inverse, le muscle IF présente une oxydation élevée de la myoglobine combinée à des valeurs de SR-TBA et carbonyles importantes. La viande IF est rejetée par 1 consommateur sur 2 dès le 3ème jour de conservation. Conservés sous vide, ces deux muscles se comportent de manière identique : une stabilité de la couleur et l'absence d'oxydation.

ABSTRACT

During the last years an increase in the ratite meat production (ostriches, emus and rheas) has been observed. The meat of rhea is lean and red and its taste is similar to that of beef meat. Currently, the only studies undertaken on the rhea's meat quality relates to the sanitary and compositional aspects. The objective of this study was to determine the impact of packaging on the color stability and the oxidative process evolution of the rhea meat according to muscles. Eight 15 month old animals (*Rhea Americana*) were slaughtered and the carcasses cooled down for 24h *post mortem*. The *Gastrocnemius pars interna* (GN) and *Iliofiburalis* (IF) muscles were cut and preserved under: a) 4°C, air packaged (oxygen permeable film) during 5 days; b) 4°C, vacuum packaged during 28 days. The color parameters (CIELab* system), lipid oxidation (TBA-RS) and protein oxidation (carbonyls) analyses were carried out. The content of haem iron was determined at day 1 for all samples. For the samples preserved under air, a panel noted the aspect of the meat and indicated his purchase decision. The GN muscle packaged under air was characterized by greater color stability and lesser lipid and protein oxidation. The IF muscle showed higher myoglobin oxidation combined with a significant increase of TBA-RS and carbonyls. The IF meat was rejected by 1 consumer out of 2 after 3 days of storage. Under vacuum, both muscles showed high color stability and absence of oxidation.

INTRODUCTION

En Amérique du Sud, les espèces animales locales constituent d'importantes sources de protéines alimentaires. C'est le cas du Nandou (*Rhea americana*), grand oiseau de la famille des ratites qui occupe de larges superficies en Argentine, Brésil, Bolivie, Paraguay et Uruguay. Ces dernières années, le nombre d'élevage de Nandous a augmenté substantiellement dans ces pays, avec pour objectif principal la production de viande pour le marché local. Devant l'intérêt croissant au niveau international pour ces viandes exotiques, les producteurs envisagent la possibilité d'étendre le marché des exportations. A ce titre, il convient d'assurer aux consommateurs une qualité constante et optimale du produit.

Dans ce contexte, le mode de conditionnement de la viande constitue un levier déterminant en terme qualitatif. Outre la maîtrise des développements microbiens, les propriétés nutritionnelles et sensorielles doivent être optimisées. Or très peu d'études ont porté sur la viande de Nandou. Nous disposons donc de peu d'informations scientifiques à ce sujet. Les modifications *post mortem* dans les muscles incluent la diminution du système de défense antioxydant (Renerre et al., 1996 ; Renerre et al., 1999) et l'augmentation de l'oxydation des lipides et protéines (Martinaud *et al.*, 1997 ; Mercier et al., 1998). L'oxydation des lipides génère des aldéhydes à l'origine du développement de l'odeur de rance et la détérioration de la flaveur de la viande. Par ailleurs, les modifications oxydatives des acides aminés, dont certains sont essentiels, conduisent à une modification de leurs propriétés fonctionnelles et affecte leur digestibilité, ce qui conduit à une diminution de la valeur nutritionnelle des protéines (Santé-Lhoutellier et al., 2007 ; Santé-Lhoutellier et al., 2008a). De plus, l'oxydation des lipides et protéines est fortement reliée à l'oxydation de la myoglobine dans la viande (Renerre et Labadie, 1993). Il est déjà bien établi dans la littérature que la couleur de la viande fraiche est une caractéristique particulièrement importante à prendre en compte puisqu'elle va influencer directement la décision d'achat par le consommateur. Bien que le Nandou soit classé parmi les oiseaux, les caractéristiques de sa viande sont tout à fait originales et diffèrent largement de celles des viandes de poulet et de dinde, notamment par sa couleur rouge.

Cette étude a pour objectif d'évaluer la stabilité de la couleur et l'évolution des processus oxydatifs de la viande de Nandou en fonction du type de conservation (sous film perméable à l'oxygène et sous vide) et ceci dans les deux principaux muscles de la cuisse : le *Gastrocnemius pars interna* et le muscle *Iliofiburalis*.

1. MATERIELS ET METHODES

L'expérimentation a été réalisée sur 8 nandous (*Rhea americana*) des deux sexes, âgés de 15 mois en moyenne. Les animaux ont été abattus dans l'abattoir expérimental de l'INRA de Theix après un étourdissement manuel. Les muscles *Gastrocnemius pars interna* (GN) et *Iliofiburalis* (IF) ont été réfrigérés, prélevés 24 heures après l'abattage et conservés sous deux différentes conditions : 1) à 4°C sous film perméable à l'oxygène pendant 5 jours; et 2) à 4°C sous vide pendant 28 jours. Pendant la conservation, un suivi des paramètres de couleur, de l'oxydation lipidique et de l'oxydation protéique a été réalisé après 1, 3 et 5 jours de conservation sous film perméable à l'oxygène et après 7, 14, 21 et 28 jours de conservation sous vide.

La stabilité de la couleur de la viande a été déterminée par réflectométrie (coordonnées L*, a* et b* du système CIELab), avec un spectromètre Kontron Uvikon 933. Le ΔR, ou la réfléctance R630-R580, correspond à la détermination de la myoglobine oxydée. Une pastille de trois centimètres de diamètre et un centimètre d'épaisseur a été découpée à l'aide d'un cylindre en acier et placée dans le spectrophotomètre équipé d'une sphère d'intégration. Le spectre de réflectance a été effectué dans le domaine du visible (360-760 nm) contre un blanc de sulfate de baryum. Pour le conditionnement sous vide, les mesures objectives de la couleur ont été effectuées après l'ouverture de l'emballage et une oxygénation de 2 heures à l'air. Pour les échantillons sous film perméable à l'oxygène, une analyse sensorielle visuelle a été réalisée par un jury de 10 personnes qui ont noté sur une échelle numérotée de 1 à 5 la couleur (1 : Marron - 5 : Rouge et 1 : Foncée - 5 : Claire) et l'aspect de la tranche (1 : Désagréable - 5 : Agréable) et indiqué sa décision d'achat (oui ou non).

Le dosage des pigments héminiques a été réalisé selon la méthode d'Hornsey (1956) sur la viande fraîche à 1 jour *post mortem*.

La mesure de l'oxydation lipidique a été réalisée par la détermination des substances réactives à l'acide thiobarbiturique (SR-TBA), selon la méthode de Lynch et Frei (1993), modifiée par Mercier et al. (1998). Pour évaluer le taux d'oxydation des protéines du muscle au cours de la conservation, des dosages des groupements carbonyles ont été effectués selon la méthode décrite par Oliver et al. (1987).

L'analyse de variance et des corrélations a été effectuée en utilisant le logiciel SAS. Les moyennes ont été comparées par le test de Student.

2. RESULTATS ET DISCUSSION

Stabilité de la couleur : Quel que soit le mode de conservation, les valeurs de luminosité L* et d'indice de jaune b* sont équivalentes dans les deux muscles (34 et 9 en moyenne, respectivement). La Figure 1 montre que, sous film perméable à l'oxygène, l'indice de rouge a* diminue plus rapidement pour le muscle IF que pour le muscle GN. L'ANOVA révèle un effet significatif du muscle et du temps de conservation ($p<0.0001$). A J3, les valeurs de a* des muscles GN et IF sont similaires, mais à J5 elles sont

significativement différentes entre les deux muscles ($p<0.0001$). On peut même dire, qu'en terme de couleur, le muscle GN est parfaitement stable sur 5 jours, alors que l'indice de rouge chute d'environ 50% dans le cas de l'IF. En étudiant la viande d'autruche, Paleari *et al.* (1998) ont trouvé des valeurs initiales plus importantes de a* (environ 22,8). Pour le muscle de la cuisse de dinde et pour la viande bovine les mêmes auteurs ont reporté des valeurs moyennes de 19,3 et 21,7, respectivement.

En ce que concerne la stabilité de la couleur, les mesures de réflectance (ΔR) soulignent une oxydation plus importante de la myoglobine pour le muscle IF au cours de la conservation, ce qui se traduit par une coloration en surface de la viande marron. Les valeurs moyennes initiales de ΔR sont de 14,6 pour le muscle GN et 16,9 pour le muscle IF, après 5 jours de conservation, 9,4 et 2,5, respectivement. Pour la viande d'émeu, Berge *et al.* (1997) ont rapporté des résultats similaires. Au début de la conservation sous film perméable à l'oxygène, ces auteurs ont trouvé des valeurs de ΔR d'environ 11. Après trois jours cette valeur décroit de moitié et à J8 des deux tiers. L'ampleur de l'instabilité dépend cependant du muscle considéré. La viande de Nandou issue du muscle IF est beaucoup plus instable au contact de l'air par rapport au muscle GN.

L'analyse sensorielle visuelle de la viande conservée sous film perméable à l'oxygène indique qu'à partir de 3 jours de conservation, moins de 40% du jury serait favorable à l'achat de la viande issue de muscle IF ; alors que plus de 75% du jury serait favorable à l'achat de la viande GN.

La corrélation entre les valeurs de a* et l'aspect Désagréable-Agréable de la viande à J5 est élevée ($r=0,9$; $p<0,0001$). Par ailleurs, on observe que l'aspect de la viande est jugé désagréable pour des valeurs d'indice de rouge inférieures à 14. En revanche, pour la viande conservée sous vide, aucune évolution de l'indice de rouge n'est observée pendant les quatre semaines de conservation (Figure 2).

Les muscles de Nandou présentent une grande concentration de pigments héminiques, ce qui leur confère une couleur rouge similaire à celle des viandes bovine et ovine. L'effet du muscle sur la teneur en myoglobine, estimée à partir de la concentration en fer héminique, est montré dans la Figure 3. Le muscle IF présente des teneurs en fer héminique plus élevées que le muscle GN et parallèlement une plus grande instabilité de la couleur. Nos données sont en accord avec celles trouvées pour les muscles de ratites dans la littérature avec des concentrations en fer héminique de l'ordre de 26-29 $\mu g.g^{-1}$ de muscle (émeu : Patak et Baldwin, 1993 ; Berge et al., 1997 ; autruche : Heinze et al., 1986 ; Sales, 1996). Il existe une variabilité importante en fonction des muscles considérés. Ramos et al. (2009) ont trouvé des concentrations en fer héminique pour le muscle *Iliofibularis* de rhea d'environ 19,3 $\mu g.g^{-1}$ de muscle. Pour les trois muscles étudiés par ces auteurs, le muscle *Iliotibialis lateralis* a eu la plus grande quantité en fer héminique (28 $\mu g.g^{-1}$ de muscle), suivi par l'*Obturatorius medialis* (22,9 $\mu g.g^{-1}$ de muscle) et, en dernier, par le muscle *Iliofibularis.* Nos valeurs sont plus importantes pour le muscle *Iliofibularis*, mais sont similaires à la valeur moyenne reportée pour le muscle *Iliotibialis lateralis*. Chez le bœuf, la teneur en fer héminique varie entre 16 et 20 $\mu g.g^{-1}$ de viande (Berge et al., 1993 ; Ramos *et al.*, 2009), chez le poulet entre 1 et 5 $\mu g.g^{-1}$ de viande (Touraille *et al.*, 1981) et chez le canard, entre 13 et 22 $\mu g.g^{-1}$ de viande (Berge et al., 1997). Selon Renerre (1977), il y a une corrélation étroite entre la quantité de pigments héminiques musculaires et la respiration mitochondriale. La concentration en pigments héminiques est reliée au métabolisme oxydatif (Hunt et Hedrick, 1977). Ainsi, les teneurs respectives en pigments héminiques des deux muscles de Nandou suggèrent un caractère oxydatif plus prononcé du muscle IF que GN.

Oxydation des lipides et protéines : La Figure 4 montre l'évolution de l'oxydation des lipides mesurée par la teneur en SR-TBA. Les valeurs observées au départ (J1) sont très proches dans les deux muscles et comparables aux valeurs mesurées dans les viandes rouges, comme le bœuf (Gatellier et al., 2005) ou l'agneau (Santé-Lhoutellier et al., 2008b). Au cours de la conservation on observe une augmentation de la teneur en SR-TBA plus marquée dans le muscle IF que dans le muscle GN. A partir de J3 la teneur en SR-TBA du muscle IF a doublé, alors que celle du muscle GN n'a augmenté que de 40%. A J5 la différence d'oxydation entre les deux muscles est encore plus marquée, avec des valeurs de SR-TBA 4 fois plus élevées dans le cas du muscle IF. Dans la viande de bœuf, conservée dans les mêmes conditions, un doublement de SR-TBA a été observé (Gatellier et al., 2001; Gatellier et al., 2005).

L'oxydation des protéines mesurée par le taux de carbonyles montre une évolution différente dans les deux muscles. En effet, pour des valeurs initiales comparables de 2,4 $nmol.mg^{-1}$ de protéine dans les muscles GN et IF, on observe de 26% à J3 à 60 % à J5 d'augmentation du taux de carbonyles dans les muscles IF conservés sous film perméable

à l'oxygène. L'oxydation des carbonyles dans le muscle GN n'évolue pas au cours de la même période.

L'absence de données sur les viandes de ratites ne nous permet pas de comparer nos résultats d'oxydation protéique. Cependant dans le muscle *Sartorius* de dinde, Mercier *et al*. (1998) ont rapporté des teneurs en carbonyles maximales d'environ 3,15 nmol.mg^{-1} protéines après 9 jours de conservation. Pour la viande de bœuf, les valeurs publiées par Insani *et al*. (2008) sont encore plus faibles. À J9 ces auteurs ont trouvé une faible augmentation de taux de carbonyles de la viande de bœuf et les valeurs finales ont été toujours inférieures à 2 nmol.mg^{-1} de protéines.

Les radicaux générés pendant l'oxydation des lipides, associés à la présence des ions métalliques, favorisent l'oxydation protéique. Ainsi, après 5 jours de conservation sous film perméable à l'oxygène, les viandes présentant des valeurs de SR-TBA importantes, se caractérisent également par une production élevée de metmyglobine et de carbonyles. La corrélation entre taux de carbonyles et oxydation des lipides à la fin de la conservation sous film perméable à l'oxygène est élevée ($r=0,854$; $p<0,0001$). Une corrélation inverse ($r=-0,807$) et significative ($p=0,0002$) entre les valeurs de SR-TBA et d'oxydation de la myoglobine (ΔR) a aussi été mise en évidence, ainsi qu'entre carbonyles et l'oxydation de la myoglobine ($r=-0,722$, $p=0,0016$).

Le conditionnement sous vide se révèle particulièrement adapté à la conservation de la viande de Nandou, quel que soit le muscle considéré. En effet, la viande a montré une grande stabilité oxydative (résultats non montrés). Pendant toute la période de conservation, nous n'avons pas observé de variations significatives des valeurs de SR-TBA et de carbonyles, qui sont restés proches des valeurs moyennes respectives de 1.5 mg de MDA.kg^{-1} de viande et 2.5 nmol.mg^{-1}de protéines.

CONCLUSION

Selon le mode de conservation, les muscles de Nandou se comportent différemment : la conservation sous film perméable à l'oxygène est particulièrement préjudiciable à l'acceptabilité de la viande et ce en raison des oxydations lipidiques et protéiques dont est le siège le muscle IF et dans une moindre mesure le muscle GN. La conservation sous vide, en limitant ces phénomènes oxydatifs, semble appropriée aux deux muscles. Des travaux complémentaires sont à envisager afin de comprendre les mécanismes à l'origine de cette accélération des phénomènes oxydatifs dans le muscle IF.

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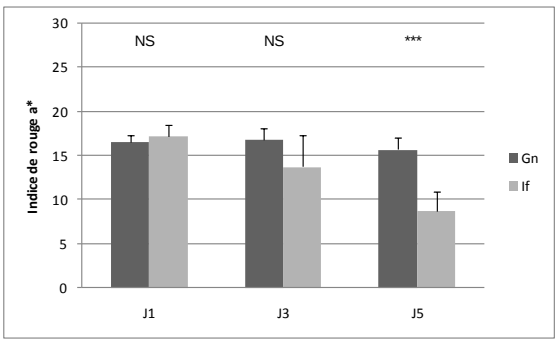

Figure 1. Evolution de a* pendant la conservation sous film perméable à l'oxygène des muscles *Gastrocnemius pars interna* (Gn) et *Iliofiburalis* (If) de nandou (*Rhea americana*) à 4°C

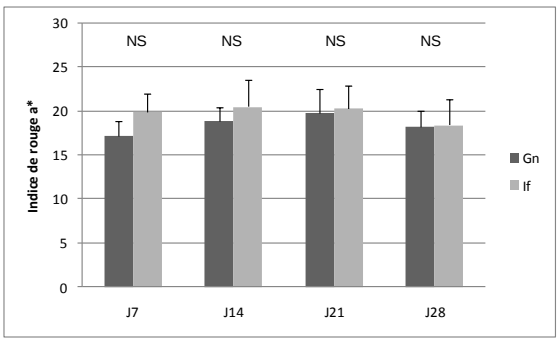

Figure 2. Evolution de a* pendant la conservation sous vide des muscles *Gastrocnemius pars interna* (Gn) et *Iliofiburalis* (If) de nandou (*Rhea americana*) à 4°C

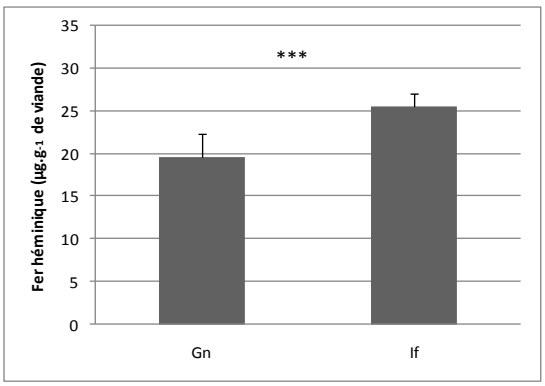

Figure 3. Teneur en fer héminique des muscles *Gastrocnemius pars interna* (Gn) et *Iliofiburalis* (If) de nandou (*Rhea americana*)

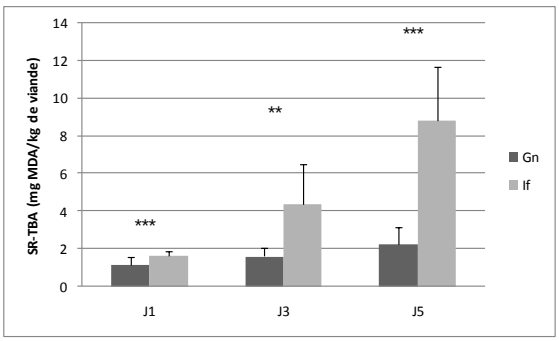

Figure 4. Evolution de la teneur en SR-TBA pendant conservation sous film perméable à l'oxygène des muscles *Gastrocnemius pars interna* (Gn) et *Iliofiburalis* (If) de nandou (*Rhea americana*) à 4°C

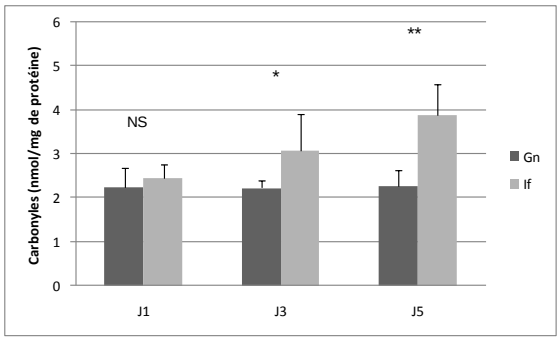

Figure 5. Evolution du taux de carbonyles pendant la conservation sous film perméable à l'oxygène des muscles *Gastrocnemius pars interna* (Gn) et *Iliofiburalis* (If) de nandou (*Rhea americana*) à 4°C.