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Effect of frozen storage duration and cooking on physical and oxidative changes in *M. Gastrocnemius pars interna* and *M. Iliofiburalis* of *rhea americana*

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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 the 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 parameters (L*a*b*), 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 and redness a*. During storage, muscle IF was more prone to lipid and myoglobin 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.

1. Introduction

There has recently been a surge in the number of studies addressing ratite meat products and production. South Africa, which produces 214,000 of the 300,000 produced ostriches worldwide, remains the main supplier of ratite products. Most studies have focused on production yield (Morris et al., 1995ab) and meat quality, highlighting the chemical composition and nutritional value of ratite meat (Berge, Lepetit, Renerre, & Touraille, 1997; Filgueras et al., 2010; Pegg, Amarowicz, & Code, 2006; Sales, 1996, 1998; Sales et al., 1998, 1999). Ratites (ostrich, emu and rhea) are sources of proteins and minerals for human diet, and ratite meats are perceived and marketed as healthy due to their low intramuscular fat and cholesterol contents (Fisher, Hoffman, & Mellet, 2000; Paleari, Corsico, & Beretta, 1995; Sales et al., 1999; Saadoun & Cabrera, 2008). In addition, intramuscular ratite fat has a high polyunsaturated fatty acid (PUFA) content (Girolami et al., 2003; Sales, 1998; Sales et al., 1999). Not only amount of fat but also fat quality play important roles in human health. Saturated fatty (SF) acids contribute to heart disease by raising plasma low-density lipoprotein cholesterol, while PUFA reduce the risk of heart disease by lowering it (Aaslyng, 2009). Moreover, ratite muscle is rich in iron and haem iron (Lombardi-Boccia, Martínez-Domínguez, Aguzzi, & Rincón-León, 2002; Ramos, Cabrera, Del Puerto, & Saadoun, 2009), which confer the meat its red coloring and similar taste to beef.

However, while the composition of ratite meat offers advantages and health benefits, it also creates susceptibility to oxidation which quickly deteriorates meat quality (Filgueras et al., 2010). Storage and cooking are essential steps to achieving a safe and palatable product, but depending on the conditions applied, they can accelerate the oxidation processes that damage meat quality and nutritional value. As ratite production is essentially localized to specific countries (ostrich in South Africa, emu in Australia, rhea in Brazil, Argentina and Uruguay), the meat product exports generally require long storage. Freezing can extend storage from a food safety standpoint but does not completely prevent oxidation (Stika et al., 2007). Oxidative processes are complex reactions initiated from meat pigments, different classes of lipids, and proteins, and forming a variety of oxidation products. Lipid oxidation is known to be one of the major causes of deterioration in the quality of meat and meat products, and can occur in either the stored triglycerides or tissue phospholipids. Haem pigments have been implicated as the major pro-oxidant in tissue lipid oxidation (Love, 1983; Renerre & Labadie, 1993). Lipid oxidation accelerates in the immediate post-slaughter period and during handling, processing, storage and cooking, producing discoloration, drip losses, off-odor, off-flavor, texture defects, and potentially toxic compounds (Morrissey, Sheehy, Galvin, & Kerry, 1998; Richards, Modra, & Li, 2002). In meat, lipid oxidation and myoglobin oxidation are coupled, and both reactions appear capable of influencing each other (Faustman, Yin, & Nadeau, 1992; Yin & Faustman, 1993). Oxymyoglobin oxidation leads to the metmyoglobin and H_2O_2 required for lipid oxidation (Chan, Faustman, Yin, & Decker, 1997), whereas aldehyde lipid oxidation products alter myoglobin redox

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stability, resulting in the promoted oxidation of oxymyoglobin and the formation of adduct with myoglobin through covalent modification (Lynch & Faustman, 2000).

Alongside lipid and myoglobin oxidation, protein oxidation is also responsible for many biological modifications (Decker, Xiong, Calvert, Crum, & Blanchard, 1993), such as protein fragmentation or aggregation, decreased protein solubility, and decreased amino acid bioavailability (Santé-Lhoutellier, Astruc, Marinova, Grève, & Gatellier, 2008). Active oxygen species attack the side chain of basic amino acids (lysine, histidine, and arginine) and can convert them into carbonyl derivatives. These carbonyl groups can react with free amino groups to form amide bonds. Oxidative processes acting on proteins can also decrease thiol groups by forming disulfide bridges. Aromatic amino acids may also become oxidized (Martinaud et al., 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 rather than chilled products carries the advantages of increased storage time, greater inventory flexibility, and greater product control (Pietrasik & Janz, 2009). However, freezing and frozen meat storage can affect the structural and chemical properties of muscle foods and influence meat quality attributes such as thawing loss, color, and tenderness (Farouk & Swan, 1998; Honikel, Kim, Hamm, & Roncales, 1986). It is well established 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 consequently consumer acceptance (Pietrasik & Janz, 2009).

The aim of this study was to evaluate the effect of frozen storage and cooking on the physical parameters and oxidation stability of two rhea muscles: M. gastrocnemius pars interna (GN) and M. Iliofiburalis (IF). The meat was maintained frozen at −20 °C for up to 180 days before cooking at 100 °C for 30 min.

2. Materials and methods

2.1. 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 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. Carcasses were maintained at room temperature (15 °C) for 1 h 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 later determination of lipid and protein oxidations. The additional 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 to evaluation, samples were removed from frozen storage and held under darkness for 24 h at 4 °C.

2.2. Thawing loss

Frozen samples of muscles were removed from their packaging, weighed, placed in a tray and thawed at 4 °C for 24 h. 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] × 100).

2.3. Meat cooking

Samples in triplicate (5 g) 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.

2.4. 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] × 100).

2.5. 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).

2.6. Lipid oxidation

Lipid oxidation was measured by the thiobarbituric acid-reactive substances (TBA-RS) method, according to Lynch and Frei (1993) and modified for meat samples by Mercier, Gatellier, Viau, Remignon, and Renerre (1998). Results were expressed as mg of MDA per kg of meat (TBA units).

2.7. 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, and Stadtman (1987) modified for meat samples by Mercier et al. (1998). Results were expressed as nanomoles of DNPH fixed per milligram of protein.

2.8. Aromatic amino acids content

The aromatic amino acids (phenylalanine, tyrosine and tryptophan) were determined by UV derivative spectroscopy according to the method reported by Gatellier, Kondjoyan, et al. (2009). All measurements were performed in duplicate and values expressed as percentage of amino acid in fresh tissue (g/100 g meat).

2.9. 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.