Meat Science 84 (2010) 706–710

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

Oxidative status, *in vitro* iron-induced lipid oxidation and superoxide dismutase, catalase and glutathione peroxidase activities in rhea meat

A. Terevinto [a], A. Ramos [a,b], G. Castroman [a], M.C. Cabrera [a,b], A. Saadoun [a,*]

[a] Sección Fisiología and Nutrición, Facultad de Ciencias, Universidad de la República, Calle Igua, Montevideo 4225, Uruguay
[b] Dpt. Producción Animal and Pasturas, Laboratorio Calidad de Alimentos, Facultad de Agronomía, Universidad de la República, Garzón, Montevideo 809, Uruguay

ARTICLE INFO

Article history:
Received 3 March 2009
Received in revised form 2 November 2009
Accepted 9 November 2009

Keywords:
Rhea meat
Protein oxidation
Lipid oxidation
Superoxide dismutase
Catalase
Glutathione peroxidase

ABSTRACT

Rhea (*Rhea americana*) muscles *Obturatorius medialis* (OM) *Iliotibialis lateralis* (IL) and *Iliofibularis* (I), obtained from farmed animals, were evaluated regarding their oxidative/antioxidant status. The mean level of thiobarbituric acid reactive substances (TBARS) expressed as malonaldehyde (MDA) content was of 0.84 mg MDA/kg wet tissue for the three muscles. TBARS level was significantly higher in IL than OM and I, with the two latter showing similar levels. The mean level of carbonyl proteins expressed as dinitrophenylhydrazine (DNPH) was 1.59 nmol DNPH mg^{-1}. Carbonyl protein levels were significantly different ($P < 0.05$) between the three muscles (IL > OM > I). Iron-induced TBARS generation was not significantly different between the three muscles at any time, nor for each muscle during the 5 h of the experiment. Superoxide dismutase activity in IL muscle was significantly higher ($P < 0.05$) than in I muscle. However, the difference between IL and OM muscles was not significant. The differences between the three muscles became not significant when the results were expressed by mg of protein contained in the extract, instead by g of wet tissue. No differences were found for catalase (μmol of discomposed H_2O_2 min^{-1} g^{-1} wet tissue or by mg of protein contained in the extract) and glutathione peroxidase (μmol of oxidized NADPH min^{-1} g^{-1} of wet tissue or by mg of protein contained in the extract) activities between the three muscles.

1. Introduction

Rhea (*Rhea americana*) or ñandu is a flightless bird native to South America (Saadoun & Cabrera, 2008; Sales, 2006). In Uruguay, rhea are farmed to produce essentially meat, but also feather, oil and skin. To help producers promote their products in the local and international market, scientific information on quality and characteristics for this meat are needed (Saadoun & Cabrera, 2008). Information is available on the fatty acid composition of the meat (Romanelli, Trabuco, Scriboni, Visentainer, & de Souza, 2008; Sales et al., 1999), oil characteristics (Grompone, Irigaray, & Gil, 2005), mineral content of the meat (Ramos, Cabrera, Del Puerto, & Saadoun, 2009) and meat technological parameters (Sales et al., 1998). However, information concerning the oxidative/antioxidant status of rhea meat is not known.

Oxidative changes are the main non-microbial causes of quality deterioration of meat (Descalzo et al., 2005; Martinaud et al., 1997; Mercier, Gatellier, Viau, Remignon, & Renerre, 1998; Xiong et al., 2007). Lipid oxidation in muscle foods is initiated by several components arising from both internal and external sources. For example, during handling, processing and storage of fresh meat, endogenous iron is partially responsible for the catalysis of lipid oxidation, formation of rancid odours and other off-flavours (Chen, Pearson, Gray, Fooladi, & Ku, 1984; Descalzo et al., 2005; Gatellier, Mercier, Juin, & Renerre, 2005; Renerre & Labadie, 1993).

Protein oxidation implies that amino acid residues are modified as a direct consequence of oxidative damage, leading to the loss of sulphydryl groups, the generation of oxidized derivatives such as protein carbonyls and the formation of cross-links and aggregates. Protein oxidation is responsible for many biological modifications, such as protein fragmentation or aggregation and decrease in protein solubility which affects the quality of meat and meat products. Oxidation might also play a role in controlling proteolytic activity of enzymes and could be linked to meat tenderness (Mercier, Gatellier, & Renerre, 2004).

However, endogenous antioxidant factors, such as enzymes, control the oxidation in muscle tissues. Enzymes, such as superoxide dismutase (SOD), catalase and glutathione peroxidase (GPx), can counteract the meat oxidation (Daun & Åkesson, 2004). SOD and catalase are coupled enzymes. SOD scavenges superoxide anion by forming hydrogen peroxide and catalase safely decomposes

* Corresponding author. Address: Sección Fisiología and Nutrición, Facultad de Ciencias, Universidad de la República, Calle Igua, Montevideo 4225, Uruguay. Tel.: +598 2 5258619; fax: +598 2 5258617.
E-mail addresses: asaadoun@fcien.edu.uy, asaadoun_alt@yahoo.fr (A. Saadoun).

0309-1740/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2009.11.007

hydrogen peroxide to water and superoxide anion. GPx can decompose both hydrogen peroxide and lipoperoxides formed during lipid oxidation (Gatellier, Mercier, & Renerre, 2004).

The aims of the present study were; (1) to quantify and compare in three muscles of farmed rhea the oxidation status by measuring the thiobarbituric acid reactive substances (TBARS) and the carbonyl proteins (2) to quantify and compare the antioxidant defense system in the three muscles, by measuring the *in vitro* iron-induced oxidation of lipids and the activities of SOD, catalase and GPx.

2. Materials and methods

2.1. Animals and samples

The animals ($n = 6$) which were bought from a commercial breeder, were reared in a semi-intensive system with outside access located in Canelones (Uruguay). The commercial food offered was based on corn, soybean meal and pelleted lucerne containing 18% crude protein and 12 MJ kg^{-1}. Food and water were offered *ad libitum*. The animals, aged between 10 and 12 months, were slaughtered in an authorized abattoir and the muscles *Obturatorius medialis* ($n = 5$), *Iliotibialis lateralis* ($n = 5$) and *Iliofibularis* ($n = 6$), according to the rhea meat guide of Uruguay (INAC, 2003), were immediately separated from the carcass, packaged in polyethylene bags and kept frozen (2 months) in the dark at −20 °C until further analysis.

2.2. Determination of lipid oxidation

Samples of 1.5 g obtained from entire frozen muscles, were homogenized with an Ultra Turrax T18 homogenizer (IKA-Werke GmbH and Co. KG, Staufen, Germany) with 30 ml of an extraction buffer (0.15 M KCl, 0.02 M EDTA and 0.30 M BHT, at 4 °C) at 12,000 rpm for 1 min. Two 8 ml volumes of the homogenate were separated and frozen at −20 °C in two tubes for 24 h, for carbonyl protein and protein content assays. The TBARS procedure for the determination of lipid oxidation was conducted immediately after the homogenization following the methods of Lynch and Frei (1993) and Gatellier et al. (2004), with modifications. One milliliter of the homogenate was incubated with 1 ml of a 2-thiobarbituric acid (TBA)–trichloracetic acid (TCA) solution (35 mM TBA and 10% TCA in 125 mM HCl) in a boiling water bath for 30 min. After cooling in an ice bath for 5 min and standing at room temperature for 45 min, the pink chromogen was extracted with 4 ml of n-butanol and phase separation by centrifugation at 3000*g* for 10 min. The absorbance of the supernatant was measured at 535 nm. The concentration of malonhaldehyde (MDA) was calculated using the molar extinction coefficient of the MDA (156,000 M^{-1} cm^{-1}). Results were expressed as mg MDA per kg of wet meat.

2.3. Determination of protein oxidation

The protein oxidation level was determined by the carbonyl protein assay according to Mercier et al. (2004), with slight modifications. The homogenate samples, frozen 24 h before, were thawed at room temperature. Two aliquots of 2 ml from each sample were put into two different tubes. These tubes were centrifuged at 2000*g* and 4 °C for 10 min. One was incubated with 2 ml of 2 M HCl (blank) and the other one with 2 ml 0.02 M dinitrophenylhydrazine (DNPH) in 2 M HCl, for 1 h at room temperature with regular stirring. Then, 2 ml of 20% TCA was added. After stirring, the mixture was left at room temperature for 15 min with regular stirring. The tubes were centrifuged at 2000*g* for 10 min. The pellets were washed three times with 4 ml of ethanol:ethyl acetate (1:1), centrifuging each time, to eliminate traces of DNPH. The pellets were dissolved in 6 ml of 6 M guanidine HCl with 0.02 M KH_2PO_4 (pH 6.5). The tubes were incubated at room temperature for 15 min with regular stirring. Afterwards, they were centrifuged at 2400*g* for 10 min. The absorbance of the supernatant was measured at 370 nm and the concentration of DNPH was calculated using the DNPH molar extinction coefficient (22,000 M^{-1} cm^{-1}). Results were expressed as nM of DNPH per mg of protein. Protein content was determined at 280 nm in the extraction buffer using bovine serum albumin (BSA) from Sigma chemicals Co (St. Louis, USA) as protein standard, as described by Stoscheck (1990).

2.4. Determination of iron-induced lipid oxidation

Muscle samples of 2 g were homogenized in an Ultra Turrax T18 model homogenizer (IKA-Werke GmbH and Co. KG) with 20 ml of 0.15 M KCl (pH 7.2) for 1 min at 12,000 rpm in an ice bath (Gatellier et al., 2004). The homogenate was centrifuged at 2000*g* for 10 min at 4 °C and 0.5 ml of the supernatant was mixed with 0.5 ml of 0.15 M KCl, 30 μl of 3 mM BHT. Then, the procedure for TBARS assay by adding 1 ml of the TBA–TCA solution was followed. Five milliliters of the supernatant were incubated at 37 °C in a water bath, under agitation, with 5 ml of 0.5 mM $FeSO_4$ and 1 mM H_2O_2 solution (50 μl) for 30 min, 2 and 5 h. After each incubation time, 1 ml was taken and the oxidation reaction stopped by addition of 30 μl of 3 mM BHT. One milliliter of TBA–TCA solution was added and the procedure for TBARS determination was performed.

2.5. Determination of antioxidant enzyme activities

A 2.3 g sample of the frozen muscle was homogenized with an Ultra Turrax T18 (IKA-Werke GmbH and Co. KG) with 26 ml of extraction solution containing 0.15 M KCl and 0.79 M EDTA (pH 7.4) for 1 min at 12,000 rpm in an ice bath. The homogenate was centrifuged at 9000 g at 4 °C for 10 min. The supernatant was used for the determination of catalase and SOD activities.

The activity of catalase was measured by recording the H_2O_2 disappearance by the decrease in absorbance at 240 nm during 3.5 min, following the method of Aebi (1984). The incubation mixture contained 2820 μl of the extraction buffer, 90 μl of the supernatant and 90 μl of 6.56 mM H_2O_2. The activity was calculated using the molar extinction coefficient of H_2O_2 (39.4 M^{-1} cm^{-1}) and results were expressed as μM of discomposed H_2O_2 min^{-1} g^{-1} fresh meat and nM of discomposed H_2O_2 min^{-1} mg^{-1} protein.

Total SOD activity was determined as proposed by Marklund and Marklund (1974), with the modifications of Gatellier et al. (2004), by measuring the inhibition of pyrogallol autoxidation. The incubation mixture contained 2850 μl of 50 mM phosphate buffer (pH 8.2), 75 μl of the supernatant and 75 μl of 10 mM pyrogallol. The increase in absorbance at 340 nm was recorded during 2 min. One unit was taken as the activity that inhibits the reaction by 50%.

For the determination of GPx activity, 5 g of meat sample were homogenized in 25 ml of 50 mM KH_2PO_4 and 0.5 mM EDTA (pH 7.0) with an Ultra Turrax T18 model homogenizer (IKA-Werke GmbH and Co. KG) for 1 min at 18,000 rpm. The homogenate was centrifuged at 2000*g* for 2 min at 4 °C and the supernatant filtered. The assay mixture contained 50 mM KH_2PO_4 buffer, 0.5 mM EDTA, 1 mM reduced glutathione (Sigma G4251), 0.15 mM NADPH (Sigma N1630 or Fluka 93220, Fluka Chemical Co., Ronkonkoma, NY), 1.5 U glutathione reductase (Sigma G3664), 0.15 mM H_2O_2 and 1 mM NaN_3 (Sigma S-2002). The incubation mixture contained 1970 μl of the assay mixture and 30 μl of the filtered sample. The activity of GPx was measured at 22 °C recording the oxidation of NADPH by the decrease in absorbance of the incubation mixture