INFLUENCE OF MUSCLE TYPE, REFRIGERATION STORAGE AND GENETIC LINE ON ANTIOXIDANT ENZYME ACTIVITY IN RABBIT MEAT

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ABSTRACT: Oxidative processes in meat lead to meat quality deterioration. Meat has endogenous antioxidants and prooxidants. Our objective was to study the activity of the antioxidant enzymes in two different lines of rabbit and its variation through refrigerated storage. Twenty rabbits from two synthetic breeds selected for different criteria (litter size and growth rate) were used in this experiment. The activity of catalase, glutathione peroxidase (GSH-Px) and 2-thiobarbituric acid-reactive substances (TBARS) were measured during 5 days of storage at 4°C in *Longissimus dorsi* (LD) and the set of muscles of the hind leg (HL). Catalase and GSH-Px activities were higher in HL than in LD. The activity of catalase was stable during refrigerated storage for HL while decreased in LD. GSH-Px was more affected by the refrigerated storage, which decreased its activity in both muscles. No changes were shown in TBARS through the 5 days of storage. There were no differences in the activity of the antioxidant enzymes between the two genetic lines studied.

Key Words: Rabbit meat, Catalase, Glutathione peroxidase, TBARS, Genetics.

RÉSUMÉ: Les processus d'oxydations peuvent mener à la détérioration de la qualité de la viande. La viande possède des antioxydants et des pro-oxydants endogènes. Notre objectif est d'étudier l'activité d'enzymes antioxydantes dans deux souches de lapin, et leurs variations au cours du stockage en chambre froide (4°C). Vingt lapins de deux souches hybrides, choisies pour des différences sur certains critères (taille de portée et vitesse de croissance), ont été employés dans cette expérience. L'activité de la catalase, de la glutathion-peroxydase (GSH- Px) et des substances réactives à l'acide 2-thiobarbituric (TBARS) ont été mesurées pendant 5 jours de stockage à 4°C, sur le muscle *longissimus dorsi (LD)* et sur un ensemble de muscles de la cuisse (HL). Les activités de la catalase et de la GSH-Px étaient plus élevées dans le HL que dans le LD. L'activité de la catalase était stable pendant le stockage frigorifique pour HL, alors qu'elle diminuait dans le muscle LD. L'activité de GSH-Px a été affectée par l'entreposage frigorifique; son activité a diminué dans HL and LD. Aucun changement n'a été montré pour l'indice TBARS pour les 5 jours du stockage. Les deux souches de lapins ne présentent aucune différence dans l'activité des enzymes antioxydantes.

Mots Clés: lapin, viande, catalase, glutathion peroxidase, TBARS, genetique.

INTRODUCTION

Oxidative processes are involved in the development of diseases such as atherosclerosis and cancer (HALLIWELL and GUTTERIDGE, 1986; KUBOW, 1993). Living cells have several mechanisms of protection against the oxidative processes, including removal of peroxides by catalase and glutathione peroxidase (GSH-Px), the major peroxide-removing enzymes located in the cytosol (CHAN and DECKER, 1994; HALLIWELL *et al.*, 1995; DECKER and XU, 1998). Catalase is a heme-containing enzyme that catalyzes the decomposition of H_2O_2 into H_2O and O_2 (AEBI, 1983; CLAIBORNE, 1985). Removal of H_2O_2 by catalase inhibits oxymyoglobin oxidation in oxymyoglobin-liposome systems (CHAN *et al.*, 1997), prevents the formation of H_2O_2-activated metmyoglobin, that is regarded as a major factor in lipid oxidation in stored meat (HAREL and KANNER, 1985; KANNER and HAREL, 1985; RHEE, 1988), and reduces lipid oxidation in microsome systems (HAREL and KANNER, 1985; ANTON *et al.*, 1993). GSH-Px is a selenium-containing enzyme that has the ability to decrease H_2O_2, as well as a large number of organic hydroperoxides, in the presence of reduced glutathione (GÜNZLER and FLOHÉ, 1985). Several studies have shown an increase of GSH-Px activity in animals exposed to oxidative stress, as a mechanism to ward off lipid peroxidation (MARASCHIELLO *et al.*, 1999; RENERRE *et al.*, 1999).

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deterioration. Studies on meat of several species (RHEE *et al.*, 1996; PRADHAN *et al.*, 2000) have indicated that endogenous antioxidant enzymes, especially catalase and GSH-Px, could potentially delay the onset of oxidative rancidity in stored meat.

Information on antioxidant enzyme activity in rabbit meat is limited. Antioxidant enzymes activities differ between meat of different species (PRADHAN *et al.*, 2000). The activity of these enzymes could present some variation between animals of the same species. Selection of animals with high concentrations of these enzymes could increase the oxidative stability of meat.

The objective of this study was to determine the activity of antioxidant enzymes in two lines of rabbit, as well as to study the stability of the activity of these enzymes in rabbit meat during refrigerated storage, and their influence in lipid oxidation.

MATERIAL AND METHODS

Animals and preparation of meat samples

Twenty rabbits from two synthetic breeds selected for different criteria were used in this experiment. The breed V was formed by crossing two commercial dam hybrids, and was selected for litter size at weaning. The breed R was formed by crossing a commercial terminal sire hybrid with the Californian breed, and was selected on growth rate between the 4th and the 10th week of life. Animals were reared at the experimental farm of the Universidad Politécnica of Valencia. After weaning at 4 weeks of age, rabbits were placed in collective cages with eight individuals each, and fed ad libitum with a commercial pelleted diet (16% crude protein, 15.5% fibre, 3.4% fat) until slaugther.

Rabbits were slaughtered at 2 kg live weight at an abattoir located at the farm, thereby avoiding stress caused by transportation. Hot carcasses were put in a ventilated area for 1 h, and were then stored at 3-5 °C for 24 h. *Longissimus dorsi* (LD) and the set of muscles of the hind leg (HL) were dissected from the carcasses 24 h postmortem.

Each muscle was ground and divided into three batches and placed onto petri dishes (diameter = 4 cm, height = 1.5 cm) and flattened. Each petri dish was over-wrapped with oxygen-permeable polyvinyl chloride film and stored at 4 °C for 0, 2, or 5 days. At each time of storage, samples were analyzed immediately.

Assays of antioxidant enzymes

A 5-g muscle sample was homogenized in 25 ml of phosphate buffer (0.05M, pH = 7) and centrifuged at 4 °C for 2 min at 7000 x *g*. The supernatant fraction was filtered through glass wool and used to determine catalase (EC 1.11.1.6) and GSH-Px (EC 1.11.1.9) activities.

Catalase activity assay was performed as described by AEBI (1983) and MEI *et al.* (1994). The supernatant (0.1 ml) was reacted at room temperature (~22 °C) with 2.9 ml of 30 mM H_2O_2 in phosphate buffer, and the reaction (H_2O_2 loss) was monitored by measuring the absorbance at 240 nm during the initial 30 s. One unit (U) of catalase was defined as the amount of extract needed to decompose 1 mmole of H_2O_2 per min.

GSH-Px activity was determined by measuring the oxidation of NADPH at 22 °C (DE VORE and GREENE, 1982; GUNZLER and FLOHE, 1985). The assay medium (3 ml) consisted of 1 mM of reduced glutathione, 0.15 mM NADPH, 0.15 mM H_2O_2, 40 mM potassium phosphate buffer (pH =7), 0.5 mM EDTA, 1 mM NaN_3, 1.5 units of glutathione reductase, and 300 ml of muscle extract. Absorbance at 340 nm was recorded over 3 min. An extinction coefficient of 6300 M^{-1} cm^{-1} was used for calculation of NADPH

concentrations. One unit of GSH-Px was defined as the amount of extract required to oxidize 1 mmole of NADPH per min at 22 °C.

Determination of lipid oxidation

The extent of lipid oxidation was assessed by measuring 2-thiobarbituric acid-reactive substances (TBARS) using the method outlined by RAHARJO *et al.* (1992). The standard used was 1,1,3,3-Tetramethoxypropan. Results were expressed as mg malonaldehyde (MDA) equivalent/kg sample.

Statistical analysis

The data were analyzed using a mixed model where the animal was considered as a random effect. Storage time, muscle type and genetic line were considered as fixed effects. The following mixed model was used:

$$y=\mu+M+T+G+M*T+M*G+T*G+M*T*G+c+e$$

where μ is the general mean, M the effect of muscle type (fixed), T is time of storage (fixed), G is the genetic line (fixed), c is the effect of rabbit (random) and e the residual (random). The PROC MIXED Procedure of the SAS (1997) software was used.

RESULTS AND DICUSSION

Table 1 shows catalase and GSH-Px activities for LD and HL muscles. The activity of catalase showed lower values than in beef, pork and chicken thigh, although higher values than in chicken breast (PRADHAN *et al.*, 2000). Conversely, rabbit meat exhibited a high activity of GSH-Px compared with pork meat (around 0.2 U/g; LEE *et al.*, 1997; HERNÁNDEZ *et al.* 2002a). It is possible that this higher activity of GSH-Px compensates for the lower activity of catalase in rabbit meat.

Catalase and GSH-Px activities where higher in HL than in LD (Table 1). The metabolic type of muscle may influence the activity of the antioxidant enzymes. Several authors have found higher antioxidant activities in oxidative muscles than in glycolytic muscles (RENERRE *et al.* 1996, in beef; RENERRE *et al.* 1999, in turkey; PRADHAN *et al.*, 2000, in chicken; HERNÁNDEZ *et al.* 2002, in pork). Concerning TBARS, there was no difference with the muscle type.

Changes in antioxidant enzyme activities during refrigerated storage of each muscle are shown in Table 2. The activity of catalase was stable during refrigerated storage for HL, while in LD catalase activity decreased significantly between 0 and 2 days with no differences between 2 and 5 days. Previous studies have indicated the stability of catalase in refrigerated chicken (RENERRE *et al.*, 1996), beef *semimembranosus* and pork muscle (PRADHAN *et al.*, 2000 and HERNÁNDEZ *et al.*, 2002a). However, other studies (RENERRE *et al.*, 1999) indicate that the stability could be related with the type of muscle. Our study has confirmed the stability of catalase in refrigerated rabbit meat, with no changes in HL and only a decrease of 17% in LD during the five days of refrigerated storage. The stability of catalase during frozen storage has also been reported in pork, beef and chicken muscles (LEE *et al.*, 1997; PRADHAN *et al.*, 2000).

GHS-Px activity decreased significantly over storage days in LD and HL (Table 2). There was a significant decrease between 0 and 2 days (42% and 40% of decrease for LD and HL, respectively) with no changes between 2 and 5 days of storage. These results differ from those of fish (WATANABEE *et al.*, 1996) and several beef muscles (RENERRE *et al.*, 1996), in which there was a stability of GSH-Px. However, our results are in agreement with those on turkey (RENERRE *et al.*, 1999) and pork *longissimus dorsi* (HERNÁNDEZ *et al.*, 2002a). There is no information about stability of catalase and GSH-Px in rabbit meat during refrigerated storage. On the other hand, the decrease in the

Table 1: Effect of muscle type on catalase and glutathione peroxidase (GSH-Px) activities and TBARS values.

Muscle	Catalase (U/g)	GSH-Px (U/g)	TBARS (mg MDA eq. /kg)
LD	186[a]	0.858[a]	0.509
HL	279[b]	0.933[b]	0.501
S.E.	7	0.025	0.010

TBARS: thiobarbituric acid substances, MDA: malonaldehyde, LD: *Longissimus dorsi*, HL: the set of muscles of the hind leg.
Means in the same column with different superscripts differ significantly ($P< 0.05$).
S.E.: standard error of the differences between muscles.

antioxidant enzyme activity had not effect on the level of lipid oxidation since the TBARS value was stable during the storage period with values around 0.5 mg MDA/kg of meat. The decrease in catalase activity was not very high and the residual GSH-Px activity seems high enough to control the oxidation.

Variations in the activity of the antioxidant enzymes between different genetic types could lead to differences in oxidative stability of the meat. There is evidence in pork meat on the influence of genetics in the activity of catalase (HERNÁNDEZ *et al.*, 2002b), but there is no information on rabbit meat or other species. In our study, no differences were found between the rabbit lines studied.

In conclusion, our results indicate that the activity of catalase and GSH-Px in rabbit meat could have an important role in controlling lipid oxidation, especially GSH-Px, due to the high activity of this enzyme in rabbit meat when compared with other species. The activity of the antioxidant enzymes in rabbit meat seems enough to control oxidation during the storage period, despite the decrease of the activity between 0 and 2 days of refrigerated storage. No evidence of genetic differences in antioxidant enzymes activity

Table 2: Storage time effect on catalase and glutathione peroxidase (GSH-Px) activities.

Muscle	Storage time (days)	Catalase (U/g)	GSH-Px (U/g)
LD	0	215[a]	1.196[a]
	2	177[b]	0.693[b]
	5	167[b]	0.687[b]
	S.E.	12	0.045
HL	0	272	1.196[a]
	2	287	0.799[b]
	5	274	0.805[b]
	S.E.	12	0.043

LD: *Longissimus dorsi;* HL: set of muscles of the hind leg.
Within muscle, means in the same column with different superscripts differ significantly ($P< 0.05$).
S.E.: standard error of the differences between treatments.

was found in rabbit meat, thus no evidence of genetic variability between breeds was found for these enzymes.

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