Antioxidant enzymes activity in *Psoas major* beef muscle from different production systems

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Background
Lipid oxidation is one of the primary factors that causes quality deterioration in meat and meat products during storage. Since generation of reactive oxygen species (ROS, free radicals) induces oxidation of membrane phospholipids and the resulting lipoperoxidation products intervene in radical propagation chain, this step is critical in oxidation development. On the other hand, lipophilic and hydrophilic molecules and antioxidant enzymes that scavenge ROS constitute the cellular antioxidant defence.
The primary antioxidant enzymes (AOE) that reduce the oxygen radical cascade are superoxide dismutase (SOD), that scavenges superoxide anion by forming hydrogen peroxide, and catalase (CAT) and glutathion peroxidase (GPx) that decompose hydrogen peroxide and lipoperoxides (Ahmad, 1995). This shows the importance of knowing the significance of the activity of AOE on refrigerated meat oxidation stability.

Objective
The aim of this work was to determine, during refrigerated retail storage, the contribution of primary antioxidant enzymes to the oxidative status of meat from steers finished on pasture or feedlot.

Material and Methods
Animals and sample preparation: Ten crossbred steers were reared on pasture. Five of them were randomly assigned to remain on this diet, and the other five were finished on feedlot system during 110 days until slaughter. The middle part of each *Psoas major* muscle was cut into 10 slices of 2.5 cm. The slices were randomly distributed among the different storage times (1, 3, 5, 7 and 9 days). Two slices of fresh meat were packaged on Polyfoam trays and overwrapped with an oxygen-permeable polyvinylchloride film. The samples were stored under simulated retail display conditions: illumination (1900 Lx) and refrigeration temperature (2 ± 1°C). (Insani et al., 2000).
Enzymatic analyses: AOE activities were determined after 1 and 9 days of exposure to retail display conditions.
SOD activity was determined by epinephrine autooxidation method according to Misra and Fridovich, (1972).
CAT activity was followed by decomposition of hydrogen peroxide at 240 nm as described by Aebi (1984).
GPx assay was determined according to Flohé and Günzler (1984)
Protein content in homogenates was determined by the Coomasie Blue method (Bradford, 1976).

Results and Discussion

After 1 day of storage, SOD activity showed no differences between pasture and grain fed animals ($p > 0.05$). And there was not significant modification of the activity during the storage period; however, a slight increase was observed for both experimental groups after day 9 of storage (Figure 1).

The results summarised in Figure 2 show the behaviour of CAT. In this case, CAT activity was higher ($p > 0.05$) for pasture finished steers meat (P) than for steers finished on feedlot meat (F). And as SOD. CAT activity remained invariable after 9 days storage. No interaction was detected at the 5% significance level among basal diet and time.

SOD and CAT protect the cells by reducing the steady–state levels of superoxide anion and hydrogen peroxide respectively. Since SOD and CAT are coupled enzymes, one possible explanation for their behaviour is that under our experimental conditions, the regulations of both enzymes might follow a similar activation pathway. Although correlation for the respective matrixes resulted low, group means showed the same tendency.

Contrarily to SOD and CAT, GPx enzyme activity was higher ($p < 0.05$) in F fresh meat samples (Figure 3). This observation support the hypothesis that more oxidation susceptible meat presents higher activation of enzymatic antioxidant defences (Hassan, 1989). Besides, unlike the other enzymes, GPx activity decreased significantly along the storage time for both P and F meat.

GPx also showed a strong negative correlation with TBARs content. Correlation coefficients of –0.88 and –0.92 were obtained for F and P matrixes respectively. De Vore and Greene (1982) previously reported similar results for GPx values over 23 nmole NADPH oxidised/mg protein/min in meat. Besides, GPx correlated positively with α-tocopherol content, with correlation coefficients of 0.87 and 0.94 for F and P basal diets (at 0.05 level). Therefore, correlation coefficients resulted highly dependent on basal diet, indicating that the activation pathway may differ according to the prooxidant–antioxidant balance in muscle tissues.

These results indicate that GPx enzyme itself is more susceptible than SOD and CAT enzymes to oxidation development during storage. In that case, it might be protected against oxidative damage by α-tocopherol. Moreover, the GPx activity decline could be related to the higher level of carbonyls determined on this samples after 9 day of storage (Insani et al., 2000). This result indicates that protein oxidation developed during storage might cause protein damage with collateral loss of functionality. Other authors also reported a significant decrease in GPX activity along storage period and a relationship with protein oxidation (Renerre et al., 1996). Moreover, GPX activity in tissue homogenates showed to be inactivated by increasing amounts of terc–butyl–hyhroperoxide, revealing its susceptibility to oxidation (data non shown).

Conclusion

The initial higher level of CAT and GPx enzymes found in feedlot beef did not contribute to reduce oxidation development after nine days of refrigerate storage. Contrarily, GPx presented a negative correlation with oxidation levels, showing to be significantly sensible to oxidising conditions developed along storage time, for both, pasture and feedlot meat.
SOD and CAT activities did not evidence a positive or negative contribution to the oxidation stability of muscle tissue under the experimental conditions of the current assay.

Literature

Ahmad, S. 1995. Antioxidant mechanisms of enzyme and proteins. In: Oxidative stress and antioxidant defenses in biology. Ed. S. Ahmad. Chapman & Hall, NY. p. 238-272.

Aebi, H. 1984. Catalase *in vitro*. In: Oxygen Radicals in Biological Systems. Methods in Enzymology. 105:121-126.

Bradford, M. M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.De Vore and Greene 1982. Glutathione Peroxidase in Post–Rigor Bovine Semitendinosus muscle. J. Food Science 47, 1406–1409.

Hassan, H. M. 1989. Attenuation of antioxidant enzymes in response to oxidative stress. Nutritional Impact of Food Processing. Bibl Nutr Dieta. Basel, Karger 43, 278–287.

Insani, M.; Eyherabide, A.; Descalzo, A.; Sancho, A. and Pensel, N. 2000. Argentine beef meat: lipid and protein oxidation and its relationship with natural antioxidants during commercial display. Proc. 46th ICoMST, Buenos Aires, Argentina.

Flohé, L. and Günzler, W. 1984. Assay of glutathione peroxidase. In: Oxygen Radicals in Biological Systems. Methods in Enzymology. 105:114-121.

Misra, H. P. and Fridowich, I. 1972 The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. J. Biol. Chem. 217: 3170–3175

Renerre M, Dumont, F and Gatellier, Ph. (1996). Antioxidant enzyme activities in beef in relation to oxidation of lipid and myoglobin. Meat Science 43: 111–121.

Figure 1: SOD activity

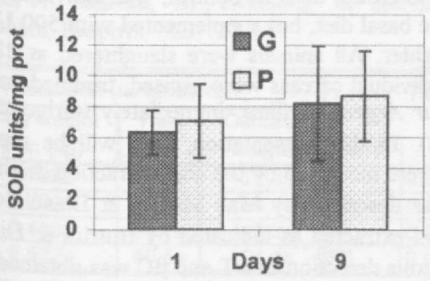
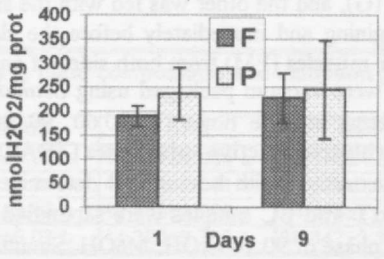


Figure 2: CAT activity



Figrure 3: GPx activity

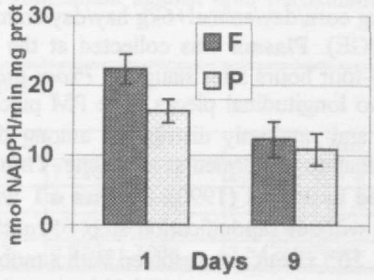


Figure 4: GPx vs. TBARs

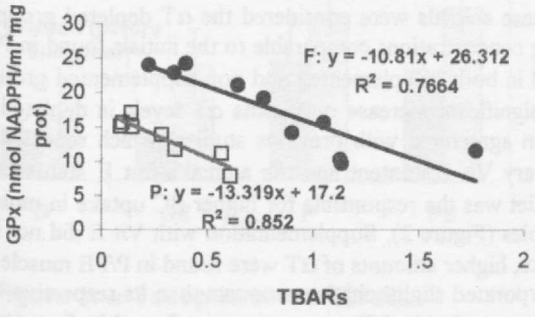


Figure 5: GPx vs. α-Tocopherol