

RELATIONSHIPS BETWEEN LIPID OXIDATION, ANTIOXIDANT ENZYME ACTIVITIES AND COLOUR STABILITY IN RAW BEEF DURING STORAGE

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Summary

The aim of this experiment was to appreciate with two bovine muscles different from the viewpoint of colour stability (longissimus thoracis and psoas major), the relationships between lipid oxidation and haeminic pigment oxidation; total iron and haeminic iron concentrations of the meat were also determined. Additionally, to underscore the production of free radicals in these reactions during meat storage, the antioxidant enzyme activities were carried out. Absorbance spectra of the meat were obtained by spectrophotometry and metmyoglobin content was measured on days 1, 3, 7, 10 and 13 storage. During the same time, lipid oxidation was followed by determination of TBA-RS. Total iron concentration was determined by atomic absorption spectrometry. Glutathione peroxidase, superoxide dismutase and catalase activities were measured on days 0 and 8 storage. After a storage of 13 days, it was shown that psoas major muscle was more color labile and displayed a greater extent of lipid oxidation than longissimus lumborum muscle; the two phenomena were highly correlated. After a storage of 8 days, there were some significant differences in antioxidant enzymic activities between muscles in relation with colour stability.

Introduction

The development of MetMb at the meat surface is depending on many biochemical factors but myoglobin autoxidation rate is one of the main responsible factor (Renerre & Labas, 1987). Although lipid and pigment oxidation are closely coupled, the relationships between the two phenomena are not always clearly established even if it is admitted that these oxidative process are of radical nature (Anton et al, 1991, 1992). During MbO₂ autoxidation, hydrogen peroxide is produced endogenously in the muscle system and is conducting to oxy-radicals; in situ, enzymic degradation of hydrogen peroxide is catalysed by catalase and glutathion peroxidase (Bendich, 1991). Moreover, if for some authors, haeminic iron is effective catalyst of lipid oxidation, others stressed that major catalytic agent would be free inorganic iron. For Faustman & Sorenson (1990), lipid oxidation is a promoter of pigment oxidation. The major objective of this study was to characterize the colour and lipid oxidation of beef from two different muscles and to quantify antioxidant enzyme activity in relation with metmyoglobin accumulation rates.

Material and methods

Two muscles different from the viewpoint of color stability and oxidative capacity : psoas major (PM) and longissimus lumborum (LL) were used. The meat was stored 1h post-mortem on Charolais cull cows (n=3) and put in liquid nitrogen. Haeminic pigment concentration was measured according to Lamand (1956). Total iron concentration in muscles were determined by atomic absorption spectrometry (Lamand et al., 1956). Colour measurements were obtained with a Uvikon 860 spectrophotometer and calculated in the CIELAB (1976) system at days 1, 3, 7, 10, and 13 storage. Metmyoglobin % at the meat surface was calculated according to Krzywicki (1979). Lipid peroxidation was measured using the thiobarbituric assay (TBA) described by Sunderman et al. (1985) with the results reported as nmoles malonaldehyde (MDA) per mg protein. Glutathione peroxidase activity was measured on days 0 and 8 storage according to Agergaard and Thode Jensen (1981). The rate of GSH reduction was monitored through the NADPH consumption as recorded at 366 nm. At the same times, SOD activity was measured according to Marklund and Marklund (1974); catalase activity was measured as described by Aebi (1974); these enzymic activities were expressed as U.A./mg protein (n=5).

Results and discussion

Between days 1 and 13 storage of beef meat, pigment and lipid oxidation for LL and PM muscles are presented in figures 1 and 2. During this period, the MetMb accumulation increased between 13 and 29% for LL muscle and between 18 and 60% for PM muscle. These results were very similar to our previous observations obtained with animals of different age, sex and breed (Renner, 1984). Intermuscular variability is effectively one of the most important factor implicated in the rate of meat discoloration in relation with fibre type profiles: Hunt & Heber (1977) have shown that PM muscle was more rich in slow-twitch oxidative fibres than LL muscle. It was established that whatever the iron was not present in greater quantities in PM muscle than in LL muscle (NS) (table 1). Haeminic iron concentrations were similar to those observed previously in our laboratory and by Bousset and Dumont (1989); expressed as percent of the total iron, haeminic iron concentrations were near of those of Hazel (1982). Moreover, as shown by Andersen & Andersen (1989) it was demonstrated that determination of iron by atomic absorption spectrometry yielded significant correlation with pigment content appreciated by Hornsey method ($r=0.785$).

For lipid oxidation, TBA tests showed an increase between days 1 and 13 storage more pronounced in PM muscle than in LL muscle. The correlation between lipid and pigment oxidation was presented in figure 3 where it could be observed a strong relationship between two phenomena (first order regression): $r=0.647$ for LL and 0.826 for PM. The correlation coefficients between these characteristics were similar to those observed on beef meat (Faustman & Cassens, 1991) or on ground veal meat (Faustman et al., 1992). Contrary to results obtained with metmyoglobin %, TBA values showed a great variability between animals probably due to the used method. Previously, Anton et al., (1991,1992). Conversely, lipid peroxides may induce MetMb formation : Mitsumoto et al. (1991) have shown that dietary vitamin E supplementation retarded metmyoglobin formation in the meat and highly suppressed lipid oxidation compared to the controls. With this meat, vitamin E supplementation has resulted in a lower myoglobin oxidation during meat storage (Santé et al, 1992).

As radical products of lipid peroxidation and myoglobin oxidation may be present, it was interesting to test the antioxidant enzyme activities present in the muscles. With the used extraction method, it couldn't be possible to discriminate between Cu-Zn SOD and Mn-SOD which catalyse the reduction of superoxide free radicals to hydrogen peroxide. For the SOD activity, no difference was found between muscles at days 0 and 8 storage for mean values (table 2) as for individual values (paired t-test) (table 3). These values were near of those found in muscles of various mammalian as cows (Sohal et al., 1990) or in rat liver (De & Darad, 1991). Moreover, by using the paired t-test, it was found that at day 0 storage, catalase activity was less important in PM muscle than in LL muscle ($P<1\%$); after 8 days storage, we observed identical results but differences between muscles are less pronounced ($P<5\%$) (table 3). As catalase activity was less pronounced in PM muscle as PM, H_2O_2 may be formed in greater quantities in this muscle; with microsomes preparations, it was observed that non-enzymatic lipid peroxidation was much more pronounced in PM muscle than in LL muscle (Anton et al. 1992).

For glutathion-peroxidase activity, there were no difference between muscles at day 0 storage but a highly significant difference at day 8 ($P<1\%$). As for catalase activity, glutathion peroxidase activity was less important in PM muscle, more instable from the viewpoint of color stability, than in LL muscle. Glutathion peroxidase activity was not far from those reported previously on heart (Sohal et al, 1990). Faustman & Cassens (1991) found that Gluteus medius muscle, more unstable than Longissimus muscle (Renner, 1984), had greater TBA values. MetMb and hypoxanthine concentration indicating a more pronounced formation of superoxide anion.

In this first experiment, if there is a strong relationship between lipid and myoglobin oxidation, conversely, it is not easy to conclude about antioxidant enzyme activities in relation with color stability.

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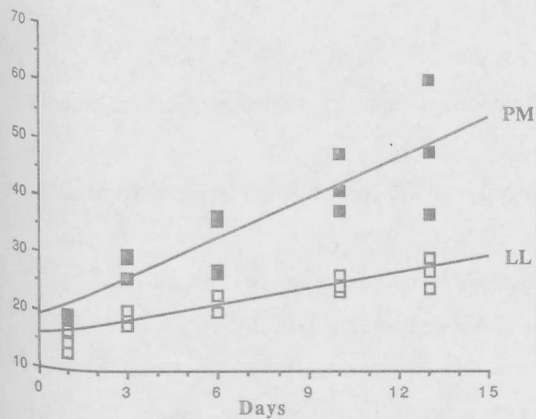


Figure 1 : Increase of MetMb % between days 1 and 13 storage

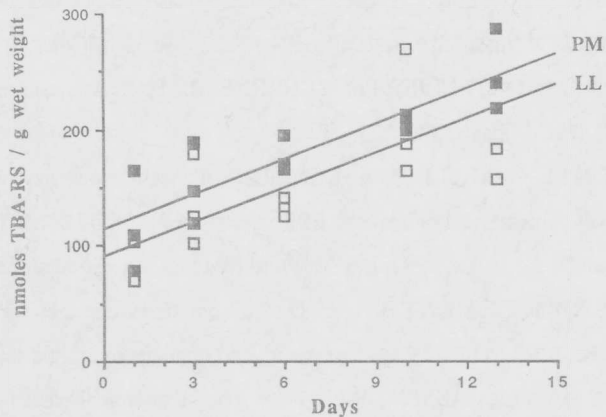


Figure 2 : Lipid oxidation between days 1 and 13 storage

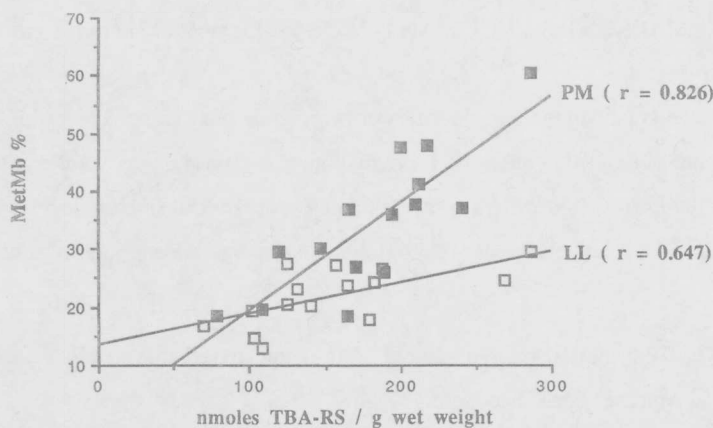


Figure 3 : Relationship between myoglobin and lipid oxidation

Muscle	Total iron (1) ($\mu\text{g/g}$)	Haeminic iron (2) ($\mu\text{g/g}$)	(2)/(1) %
LL	22.9 ± 4.3	15.7 ± 1.2	70 ± 19
PM	21.3 ± 1.0	17.1 ± 0.6	80 ± 5
	NS	NS	NS

Table 1 : Total iron and haeminic iron content of LL and PM muscles

Time	SOD		Catalase		GPx	
	LL	PM	LL	PM	LL	PM
Day 0	7.6 ± 3.3	7.3 ± 3.6	0.9 ± 0.3	0.8 ± 0.3	39 ± 14	28 ± 13
Day 8	7.7 ± 2.9	7.6 ± 2.9	0.9 ± 0.2	0.6 ± 0.2	33 ± 4	20 ± 5

Table 2 : Enzymic activities of SOD, Catalase and GPx in LL and PM muscles (mean values)

Time	SOD	Catalase	GPx
Day 0	NS	< 1 %	NS
Day 8	NS	< 5 %	< 1 %

Table 3 : differences between LL and PM for enzymic activities of SOD, Catalase and GPx (paired t-test)

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