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RELATIONSHIPS BETWEEN LIPID AND PROTEIN OXIDATION IN DIFFERENT BEEF MUSCLES

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INTRODUCTION

The development of metmyoglobin which is brown and unattractive at the meat surface is depending of many biochemical factors and oxymyoglobin autoxidation rate is one of main responsible factors implicated in colour instability. During the same time, lipid oxidation is one of the primary causes of loss of quality in meat with, particularly after cooking, the production of off-flavours and off-odours (Renerre and Labadie, 1993). To show that these oxidative processes which affect meat quality are of radical nature, we have conducted many experiments with oxymyoglobin and microsomes in model systems and precised the relationships between the lipid and myoglobin oxidation (Anton et al., 1993). If OH° and other free radicals damage cellular targets as lipids, much less is known about radical attacks on proteins. Carbonyl groups are thought to be formed after free-radical attack on amino-acid residues. The aim of this work is to better characterize the relationships between colour stability and lipid and protein oxidation during meat storage from three different beef muscles.

MATERIAL AND METHODS

Animals: Three muscles with different colour stabilities : *Longissimus Lumborum* (LL) stable, *Psoas Major* (PM) unstable and *Diaphragma* (D) very unstable (Renerre, 1984), were examined. They were taken one hour post-mortem (day 0) from 4 Friesian cows, packaged with a permeable film , stored at 4°C in darkness and analysed after 1, 3, 6, and 10 days of shelf-life.

Measurements: Colour stability was measured with a spectrophotometer equipped with an integrating sphere between 360 and 760 nm in the CIELAB system (1976). MetMb % was calculated according to the method of Krzywicki (1979). Lipid oxidation was measured by the TBA-RS method (Lynch and Frei ; 1993).

Measurement of protein oxidation was realized by the determination of protein carbonyl content where carbonyl groups were detected by reactivity with 2,4-dinitrophenylhydrazine to form protein hydrazones. The results were expressed as nmol DNPH incorporated / mg of protein (Oliver et al., 1987). The data are presented as the mean ± standard deviation with statistical significances determined by the t-test after pairing of the data.

RESULTS AND DISCUSSION

Figure 1 showed that during 10 days storage at 4°C, the MetMb % at the meat surface increased from 18 to 25 % for LL, from 21 to 32 % for PM and from 23 to 42 % for D muscle with significant differences between muscles only after a 10 days storage. This result is in good agreement with previous observations obtained from different colour-labile or -stable muscles (Renerre, 1984 ; Faustman and Cassens, 1991; Anton et al., 1993) dependent on metabolic differences. For lipid oxidation, TBA test showed an increase between day 1 and day 10 of storage (figure 2) but at day 1 the quantity of formed MDA is already important in D muscle. If for LL and PM muscles, the increase in MetMb % between days 1 and 10 was low (near of 45%), conversely the increase in formed MDA during the same time was important (between 110 and 76 %). Conversely, for D muscle in the same period, if the MetMb increase was important (83%), that of lipid oxidation was lower (35%). A strong relationship between lipid and myoglobin oxidation was observed (results not shown): r= 0.815 for LL and r= 0.767 for PM mucle according to previous results (Faustman et Cassens, 1991; Gatellier et al., 1992). It was observed earlier (Anton et al., 1991, 1992) that different radicals were implicated in these oxidative processes.

It was possible that the misuse of TBA-RS test to appreciate lipid oxidation of D muscle explained that no significant correlation between lipid and myoglobin oxidation was noted (r = 0.316). Effectively for D muscle, the quantity of lipid oxidation products decreased between day 10 and day 14 of storage (results not shown) and in this case, it would be better to determine the quantity of fluorophores which represent the end-products of the oxidative destruction of lipids (Halliwell and Gutteridge, 1989).

Protein oxidation has been measured on the total extract of the three examined muscles between day 1 and day 10 of storage. At day 1, LL and PM muscles showed a mean content of carbonyls near of 2.2 nmol/mg of protein. The content of carbonyl groups which is only one by-product resulting from oxidative attack on proteins, was higher for D muscle (2.6 nmol/mg) which is more oxidative than the other muscles but the differences were not significant (figure 3). These values were near to those found by Murphy & Decker (1989), on different muscles of chickens, and to those of Reznick et al. (1992) on Gastrocnemius muscle of rat. These last authors showed a higher content of carbonyl group in Red Quadriceps muscle of the rat (5.4 nmol/mg). Between day 0 and day 10 of storage in aerobic conditions, the carbonyl content for LL muscle increased from 2.3 to 2.9. In the same conditions, this evolution was from 2 to 4.6 for PM and from 2.6 to 5.7 for D muscle. After a 10 days storage, the differences between muscles were more evident with PM muscle in an intermediate position between LL muscle (glycolytic) and D muscle (oxidative). A significant correlation (r= 0.756; P <0.05, results not shown) was noted between carbonyl group content and MetMb % in D muscle more oxidative. Conversely, no correlation was noted between these parameters for PM and LL muscles (r = 0.228 and 0.170 respectively). For Chen and Tappel (1993) activated oxygen species produced by the presence of iron react with heme

proteins to form hemichromes which are a good indicator of oxidative damage as lipid peroxidation in animal tissues. Experiments to quantify these hemichromes during meat maturation are in progress in the laboratory.

In meat, if myoglobin is a free radical target, it also acts (in presence of H2O2) as an oxidant system versus lipids (Kanner and Harel, 1985; Anton et al; 1992) to form a porphyrin cation radical. More recent works (Davies, 1987) indicated that the oxidized proteins had a higher proteolytic susceptibility, important property to consider in the definition of meat tenderness. We had previously showed that oxidation could affect myofibrillar proteins with changes detected by ultrastructural studies (Ouali et al., 1992).

These new results indicate that protein degradation that occur in muscle during maturation, can be a sensitive indicator of cells exposure to oxygen radicals. The importance of this protein degradation in the tenderness process are in progress in the laboratory.

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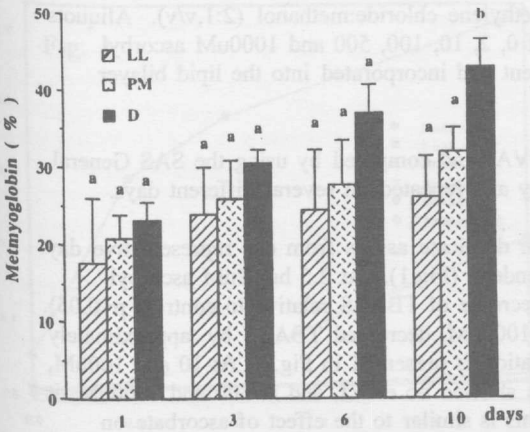

Figure 1 : Percentage of metmyoglobin formation in three different bovine muscles during 10 days storage at 4°C . (Means ± S.E.M., n=4). Values with different superscripts are significantly different (P<0.05)

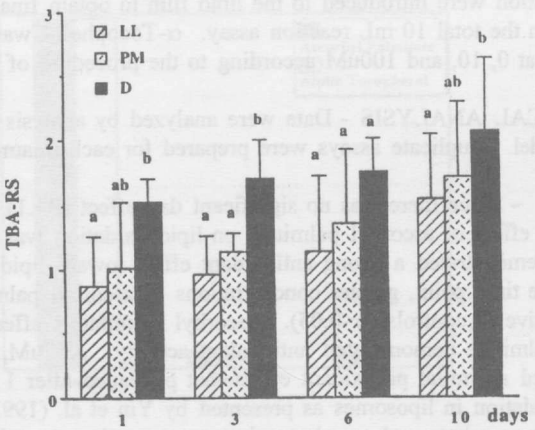

Figure 2: TBA-RS evolution in three different bovine muscles during 10 days storage at 4°C. (Means ± S.E.M., n=4). Values with different superscripts are significantly different (P<0.05)

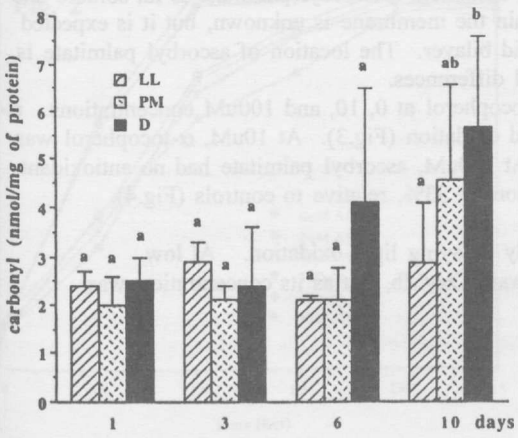

Figure 3: Carbonyl content in three different bovine muscles during 10 days storage at 4°C. (Means ± S.E.M., n=4). Values with different superscripts are significantly different (P<0.05)