COMPARISON OF DIFFERENT SYSTEMS ON LIPID AND PROTEIN OXIDATION

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INTRODUCTION

Lipid oxidation, which causes rancidity and contributes to undesirable flavour, is a major deteriorative reaction occuring in muscle foods during storage. Such oxidative changes, where oxygen and transition metals (more particularly iron) are implicated, may be initiated by nonenzymic and / or enzymic reactions (Asghar et al., 1988). If TBA-RS is well recognized and studied as lipid oxidation method, conversely, protein oxidation is not well understood (Decker et al., 1993). Among proteins, myoglobin is particularly prone to oxidation and could be considered as an indicator of oxidative stress. It was previously shown, in microsomal model systems (Anton et al., 1993) and in meat, relationships between lipid and myoglobin oxidation. In fresh meat, it was also previously observed that protein oxidation, measured by carbonyl content and / or thiol oxidation, was correlated with myoglobin oxidation during maturation (Mercier et al., 1995).

To better understand the oxidative phenomena which affect meat organoleptic qualities, it is possible to compare natural oxidative processes in meat with different iron-catalyzed oxidative systems. One of the main problems in studying these oxidative processes is that many systems exist, of an enzymic and non-enzymic nature, with different concentrations for the different components.
Stadtman (1990) has listed these different metal ion-catalyzed oxidation (MCO) systems of proteins. Many *in vitro* studies have employed Fe^{+++} and a reductant, such as ascorbate, to catalyze lipid peroxidation but the reaction of Fe^{++}/H_2O_2 is also well known. To be more reactive, iron can also be used alone as Fe^{++} but chelated with EDTA for example. For systems of enzymic nature, Xanthine oxidase, P450/P450 reductase, or different oxidase and peroxidase systems exist (Stadtman, 1990). The aim of this work was to compare 4 different oxidative systems, with varying concentration in the different components, on lipid and protein oxidation.

MATERIAL AND METHODS

From 16 weeks old turkeys, Pectoralis muscle was excised 6 h post-mortem 5 g of muscle was ground and homogenized with a Waring-blendor in 50 ml of 50 mM Tris-HCl , 100 mM KCl buffer at pH 7.4. The homogenate was centrifuged at 3000 g for 15 min and the supernatant was used to do the chemical oxidations (4 h at 37°C).

Assay for protein oxidation

Oxidative modifications of proteins were carried out using different MCO systems described in the literature (Stadtman ,1990) but with various concentrations. Three hydroxyl radical (OH°) generating systems were used:
$FeSO_4$ / EDTA and $FeCl_3$ / ascorbate as used Amici et al. (1989); $FeSO_4$ / DETAPAC / H_2O_2 as used by Fucci et al. (1983). To produce tyrosine peroxyl radicals, the mixture metmyoglobin / H_2O_2 was used (Kanner & Harel, 1985b). Carbonyl groups were detected by reactivity with 2,4 dinitrophenylhydrazine (DNPH) to form protein hydrazones as decribed by Oliver et al. (1987) with slight modifications.

Assay for lipid oxidation

Lipid oxidation was measured by the TBA-RS method as described by Lynch & Frei (1993).

RESULTS AND DISCUSSION

Considering the $FeSO_4$ / EDTA system, it was observed that with a $FeSO_4$ concentration of 4 mM, a maximum of TBA-RS and carbonyl compounds was found when the concentration was about 1mM EDTA (figures 1 and 5). With 8mM EDTA, as recommended by Amici et al. (1989), the carbonyl and TBA-RS contents were lower than those obtained when 1 mM EDTA was used. With the $FeCl_3$ + ascorbate system, the maximum value for TBA-RS was obtained with 0.1 mM ascorbate (figure 2). In the same time, it was noted that increasing ascorbate concentration to 5 mM gave the best results to follow the formation of carbonyl groups (figure 6); these results were in agreement with those of Decker et al. (1993). With this system, the concentration in different components (Fe^{+++} / Ascorbate) must be adapted to the used test but a common concentration of 0.5 mM.can be recommended for the two tests.

With the system $FeSO_4$ (4 mM) / H_2O_2, the maximum in TBA-RS and carbonyl compounds was obtained with a concentration in H_2O_2 about 8 mM (figures 3 and 7). Finally, with the system MetMb / H_2O_2, a high concentration in MetMb and H_2O_2 (600 µM) will give the best results to obtain the greater quantity in TBA-RS and carbonyl compound (figures 4 and 8). When the H_2O_2 concentration was increased from 70 to 600 µM, the carbonyl content increased from 3 to 8 nmol / mg protein (figure 8).

In conclusion, the concentrations of different components of these oxidative systems must be adapted as a function of each system to obtain the most effective study of the lipid and protein oxidation in meat.

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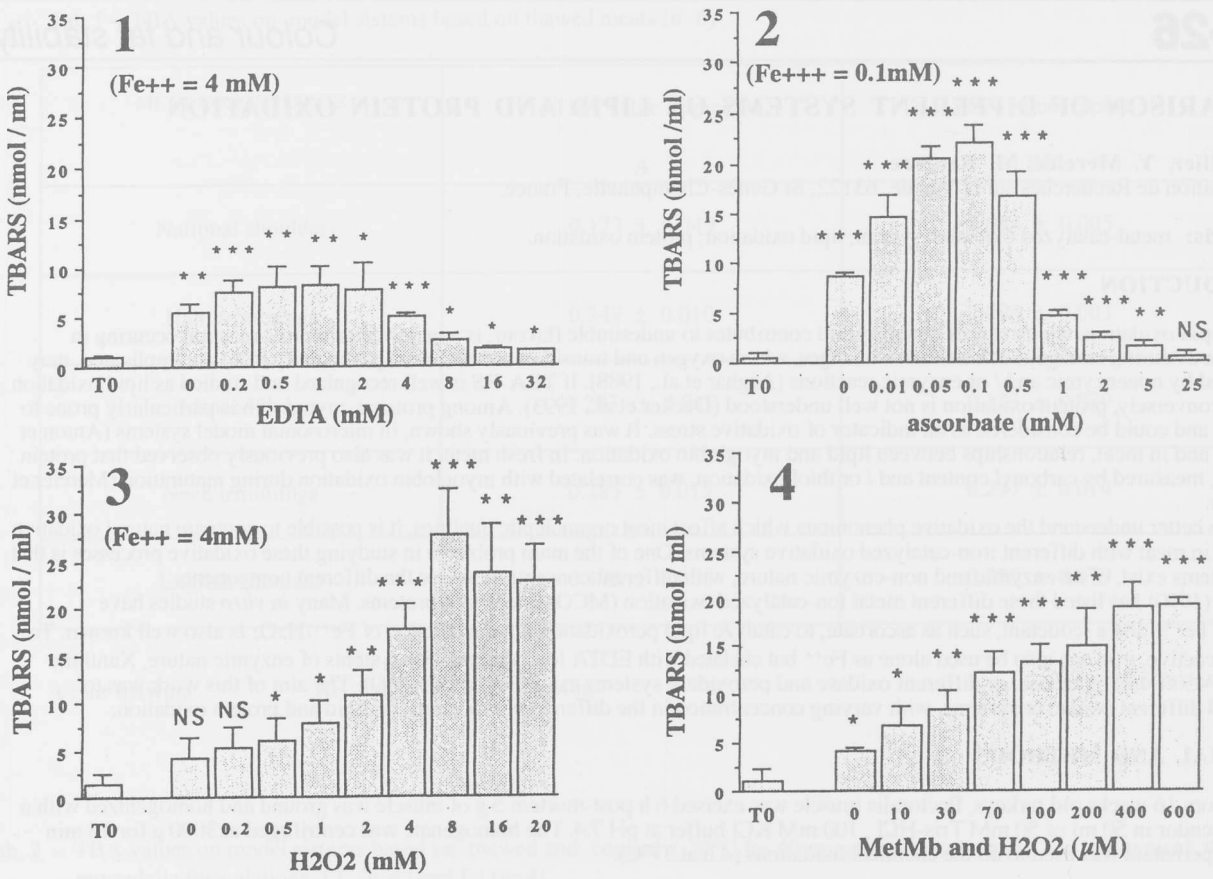
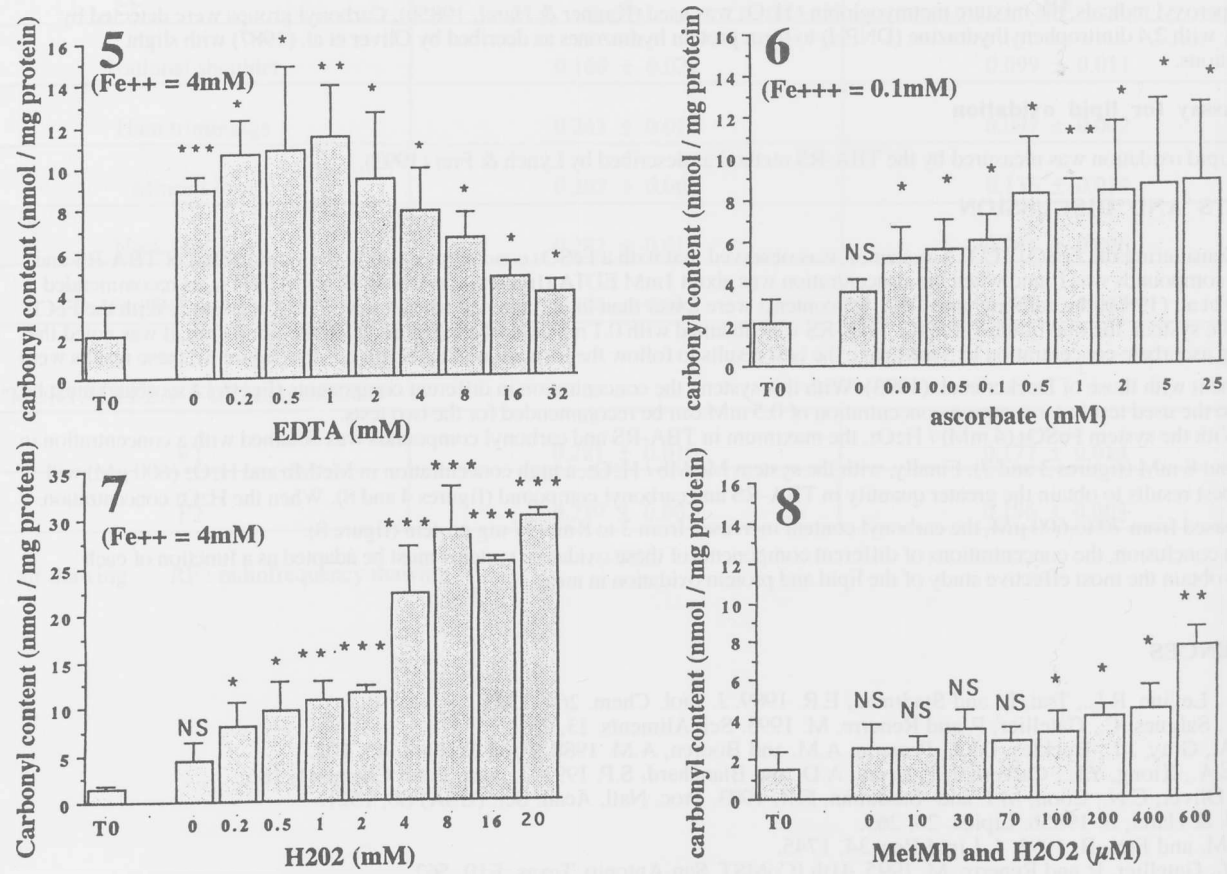


Figures 1,2,3,4: Concentration effect of different oxidative systems on TBARS production in turkey muscle homogenates. Results are expressed as means of triplicate measurements (* P<0.05, ** P<0.01, *** P<0.001).



Figures 5,6,7,8: Concentration effect of different oxidative systems on carbonyl content in turkey muscle homogenates. Results are expressed as means of triplicate measurements (* P<0.05, ** P<0.01, *** P<0.001).