N-ENZYMIC CATALYSIS OF MICROSOMAL LIPID PEROXIDATION BY ACTIVATED - TMB. DETECTION OF MYOGLOBIN-DERIVED RADICALS BY OPTICAL AND ESR CTROSCOPY.

ANTON, Ph. GATELLIER & M. RENERRE.

on de Recherches sur la viande, Theix, 63122 St Genès-Champanelle

MARY

im of this experiment was to study the non-enzymic catalysis of microsomal lipid peroxidation by "activated- metmyoglobin" leading to adical chain reaction implicated in meat quality deterioration as colour and flavor. From two bovine muscles different from the viewpoint lor stability (longissimus lumborum (LL) and psoas major (PM)), microsomes were extracted and oxymyoglobin, purified on a Mono-Q n, was transformed in metmyoglobin. After interaction of hydrogen peroxide with metmyoglobin to obtain "activated -metmyoglobin", nzymic lipid peroxidation of microsomal fraction was followed by the determination of TBA-reactive substances. Detection of radicals ecorded by optical and electron spin resonance spectroscopy.

lected by optical and ESR spectroscopy, it was noted that free-radicals were formed; myoglobin IV species may be present. The addition crosomal fraction to the mixture hydrogen peroxide-metmyoglobin gave rise to a competition between the spin -trap DMPO and branal lipid peroxides towards free-radicals species leading to a decay of ESR signal; no muscle effect was noted in these conditions. s shown that non-enzymic lipid peroxidation followed during the first two hours (TBA test) was much more prononced with microsomes cted from psoas major muscle, which is more color labile, than from longissimus lumborum muscle.

RODUCTION

ng biochemical factors which contribute to increase myoglobin autoxidation rate, free fatty acids are important factors (Stewart et al,); Genot et al, 1991). In beef meat, enzymic microsomal systems in presence of cofactors catalyse oxidation of oxymyoglobin (Anton et 991). Inversely, myoglobin has been implicated as playing an important role in catalysis of lipid oxidation (Rhee, 1988); Kanner & Harel 5) have shown that membranal lipid peroxidation was also initiated by H2O2-activated MetMb and H2O2 is endogenously produced in uscle system as during MbO2 autoxidation, process largely responsible of colour instability (Renerre, 1990).

major objective of this study was to detecte, by optical and ESR spectroscopy, if radicals were formed in the H2O2-activated MetMb en and if they could induce lipid peroxidation in presence of microsomes extracted from LL and PM muscle.

TERIEL AND METHODS

myoglobin for each bovine muscle was purified on a Mono-Q column, as described previously (Renerre et al., in press); from myoglobin, metmyoglobin was prepared by chemical oxidation with potassium ferricyanide. DMPO was purchased from Aldrich (97% ty). ESR spectra were recorded at room temperature using a BRUCKER ER 200D spectrometer under the following conditions: gain: 0$; modulation intensity : 1G.; scan time: 200s. The magnetic field was set at 3470 G. and the spectra were recorded over a 200 G. sweep e (3470 + 100 G.). Visible spectra were recorded on a Uvikon 860 from Kontron Instrument using a 10mm path-length cell. Spectra were rded in the visible from 460 to 760 nm. All solutions were prepared with distilled water passed through a Chelex 100 column to remove e metals. Phosphate buffer was also passed through the column and the chelating agent DETAPAC was added to the buffer to eliminate al traces (Xu et al. 1990). In ESR experiment, the reaction medium contained MetMb 0.6 mM, H2O2 0.6 mM, DMPO 0.1M in a 0.1 M sphate buffer at pH 7.0. Microsomes were prepared as described previously (Anton et al, 1991) and used at the concentration of 1.82 l. TBA test was appreciated , as described by Sunderman et al. (1985), at 37°C for two hours; reaction medium contained MetMb 30 H2O2 30 µM and microsomes 1mg/ml (Anton et al, 1991).

RESULTS

ESR experiments

The interaction of hydrogen peroxide with metmyoglobin conducted to the production of free radical species which could be detected by the spin trapping reagent DMPO. ESR signal consisted of six peaks located respectively at G: 3462, 3470, 3475, 3484, 3491 and 3495 G (figure 1). This signal was strictly identical to those observed by Xu et al.(1990) and Davies (1990) in similar experimental conditions. Figure 2 showed that this spin trap adduct was not stable and decayed approximately from 50% in 30 min. These results were according to those of Davies (1990) who observed that the concentration of the formed species decreased to about 10% within 10 min. In these conditions, Newman et al.(1991) have identified the final protein radical which is believed to be a tyrosine peroxyl radical together with ferryl species. In our conditions, the first results seemed indicate that ESR signal was not influenced by the muscular origin of myoglobin (LL / PM) (figure 2). The addition of microsomal membranes to the system composed of metmyoglobin + H2O2 induced an important decrease of the DMPO-ESR signal (figures 1&2). In these conditions, and after 40 min. at the end of the experiment, it was shown a decay of approximately 30% of the ESR signal comparatively to the previous assay without membrane. This result indicated that there was a competition between the spin-trap DMPO and membranal lipids towards free-radicals species; a slight muscle effect could be noted (figure 2). By addition of erythrocyte membranes, Newman et al. (1991) observed a decrease in their signal intensity which was dependent of the concentration of the membranes.

Optical spectroscopy

As shown in figure 3 , line 1 was the spectrum of MetMb ; line 2 was the spectrum of MetMb + H2O2. This spectrum consisted of three peaks at 510, 540 and 580 nm. These results were quite identical to those obtained by Davies (1990). This compound was relatively stable : when MetMb : H2O2 ratio was 1:1, the signal at 580nm decreased slowly and about 50-60% in 90 min. Addition of microsomal membranes to this system led to an increased rate of the decay of this species back to MetMb (about 70% in 90 min.) confirming the importance of this "activated-MetMb" in the initiation process of membranal lipid peroxidation (spectrum not showed). For Davies (1990), the major species present in the mixture MetMb+H2O2 was probably a tyrosine peroxyl radical ($P^{\bullet}$-Fe^{4+}= O or myoglobin IV), component which was effectively stable for a considerable period of time. This compound must not be $OH^{\bullet}$ because mannitol, an $OH^{\bullet}$ scavenger, had no effect on the extent of myoglobin IV formation. When H2O2 was used in excess with methaemoglobin (10:1), Puppo & Halliwell (1988) showed that H2O2 degraded MetHb releasing iron ions that react with H2O2 to form a species that appears to be $OH^{\bullet}$.

Lipid peroxidation

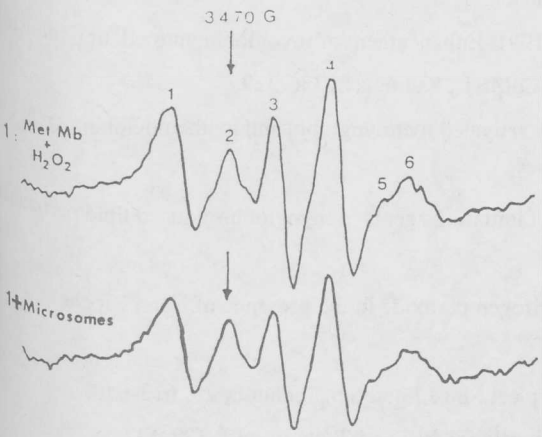
When microsomes were added to the mixture MetMb-H2O2, and after two hours, TBA-reactive material was more important in PM muscle than in LL muscle (figure 4). These results suggested that lipid oxidation by "activated-MetMb" was more prononced with microsomes of PM muscle than with microsomes from LL muscle; in parallel, differences in phospholipid composition of these membranes were noted (unpublished results). We had shown in the same time that after a meat storage of 13 days, PM muscle was more color labile and displayed a greater extent of lipid oxidation than LL muscle (Gatellier et al., 1992).

To elucidate this apparent discrepancy between TBA test and spectroscopic measurements, and to establish a muscle effect in the production of radicals in relation with colour stability, more investigations are needed. Although during meat storage, protein- and oxy-radicals must be formed , their exact nature is not always established.

REFERENCES

ANTON M., GATELLIER Ph., RENERRE M., 1991. Microsomal lipid peroxidation in relation with oxidation of bovine myoglobin. 37th ICoMST, Kulmbach, 320-323.

DAVIES M.J., 1990. Detection of myoglobin-derived radicals on reaction of metmyoglobin with hydrogen peroxide and other peroxidic



ure 1 : ESR spectra observed on reaction of MetMb + H2O2 in the presence of DMPO with or without addition of microsomes

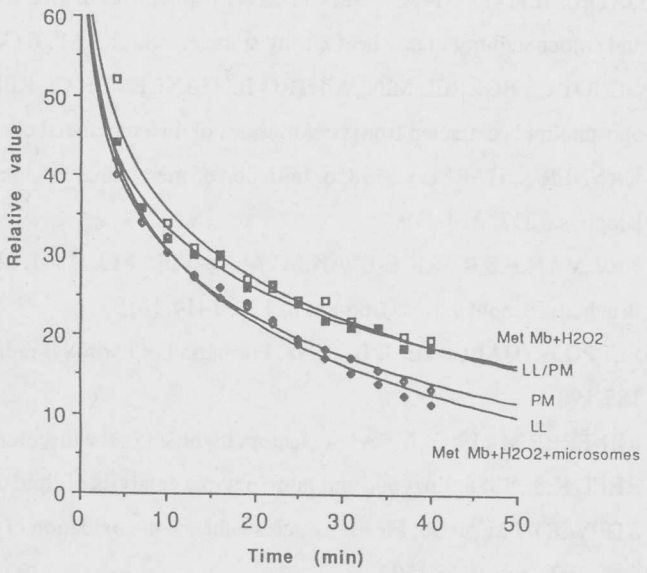


Figure 2 : Variation of the intensity of the DMPO ESR signal produced on reacting MetMb+ H2O2 with or without microsomes

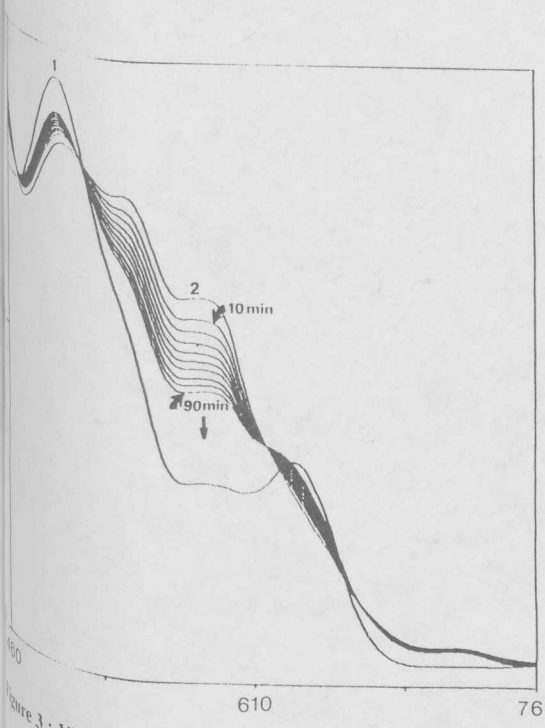


Figure 3 : Visible spectra observed in presence of MetMb (1) or MetMb + H2O2 (2)

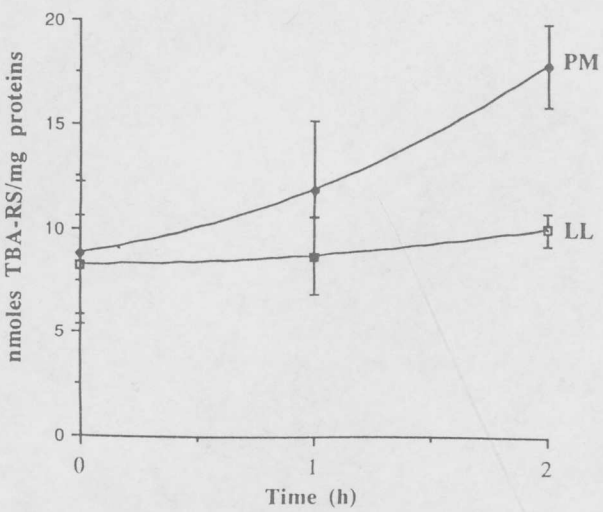


Figure 4 : TBA-test of microsomes (LL and PM) in presence of MetMb + H2O2

compounds. Free Rad.Res. Comms., 10, 361-370.

GATELLIER Ph., ANTON M., CHRAITI Fatima, RENERRE M., 1992. Relationships between lipid oxidation, antioxidant enzyme activities and colour stability in raw beef during storage., 38th ICoMST, Clermont-Ferrand.

GENOT C., BORREL M.N., METRO B., GANDEMER G., RENERRE M., 1991. Enhancement of myoglobin autoxidation induced by phospholipids extracted from beef muscles of different metabolic types. 37th ICoMST , Kulmbach, 356-359.

KANNER J., HAREL S., 1985b. Initiation of membranal lipid peroxidation by activated metmyoglobin and methemoglobin. Arch. Biochem. Biophys., 237, 314-319.

NEWMAN E.S.R., RICE-EVANS C.A., DAVIES M.J., 1991. Identification of initiating agents in myoglobin-induced lipid peroxidation.. Biochem. Biophys. Res. Commun., 179, 1414-1419.

PUPPO A., HALLIWELL B., 1988. Formation of hydroxyl radicals from hydrogen peroxide in the presence of iron., Biochem. J., 249, 185-190.

RENERRE M., 1990. A review: factors involved in the discoloration of beef meat . Int.J.Food Sci. Technol., 25, 613-630.

RHEE K.S., 1988. Enzymic and non-enzymic catalysis of lipid oxidation in muscle foods. Food Technol., 42, 127-132.

STEWART J.M., 1990. Free fatty acids enhance the oxidation of oxymyoglobin and inhibit the peroxidase activity of metmyoglobin., Cell.Biol., 68, 1096-1102.

SUNDERMAN S.W., MARZOUK A., HOPSER S.M., ZAHARIA O., REID B.S., REID M.C., 1985. Increased lipid peroxidation in tissues of nickel chloride treated rats. Annals Clin. Lab. Sci., 15, 229-236.

XU Y., ASGHAR A., GRAY J.I., PEARSON A.M., HAUG A., GRULKE E.A., 1990. ESR spin-trapping studies of free-radicals generated by hydrogen peroxide activation of metmyoglobin., J. Agric.Food Chem., 38, 1494-1497.