ANALYSIS OF FREE RADICAL PRODUCTION IN MEAT IN THE PHYSICOCHEMICAL CONDITIONS OF STORAGE AND COOKING

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Abstract –During meat processes (chilled storage, curing, cooking…), the production of free radicals (superoxide $O_2^{\circ-}$ and hydroxyl HO°), due to iron reaction with oxygen and peroxides, leads to oxidative damages on proteins and lipids. Our aim was to determine and predict the kinetic laws governing the free radical production according to the physicochemical environment. Experiments were carried out on model systems which mimic the physicochemical conditions in meats during storage or cooking (pH 6, ionic strength of 0.24 M, and temperatures varying from 4 to 75°C) using the two major oxidants in meat: Fe^{2+} and H_2O_2. Free radical production kinetics were measured for many conditions using specific probes. A stoichio-kinetic mathematical model of the Fenton process which involved a Fe(II)/Fe(III) redox oxidative cycle was elaborated. Amounts of generated free radicals were related to temperature and iron content from the experiments and the activation energy of the chemical reaction that initiate $O_2^{\circ-}$ production in biological systems was estimated at 55 kj/mol. from comparison of calculations with measurements.

Key Words: free radicals, kinetics, modelling oxidation.

I. INTRODUCTION

Meat processes (storage, cooking, curing) allow preserving or improving qualities of meat products but also lead to oxidation of lipids and proteins with a negative impact on sensory [1] and nutritional qualities [2]. Control of meat quality requires a better understanding of the mechanisms responsible of the oxidative phenomena and of the kinetic laws that govern them. The initiation stage of oxidation is crucial and characterized by the rate of reaction of oxygen and hydrogen peroxide with iron; this latter compound is more or less rich depending on muscles, animals and species. Superoxide radical ($O_2^{\circ-}$) and hydroxyl radical (OH°) are produced and initiate the cascade of reactions implicated in protein and lipid oxidations. To investigate the impact of the physicochemical parameters on the free radicals production our trials were carried out with a mimetic model of meat and a stoichio-kinetic mathematical model [3] was elaborated considering 12 interactive elementary chemical reactions. This approach enable to measure many production kinetics of $O_2^{\circ-}$ and OH°, using two specific probes (nitroblue tetrazolium and terephthalate), and to assess unknown kinetic parameters (rate constant and activation energy) by comparison of calculations to measurements. Our ultimate aim is to predict these kinetics for any meat type and any storage or cooking condition.

II. MATERIALS AND METHODS

Experiments

The reaction medium consisted of a 0.40 mM phosphate buffer at pH 6. Oxidants ($FeSO_4$ and H_2O_2) were dissolved in this medium in different concentrations (0.05 to 0.6 mM for iron and 0.2 to 2 mM for H_2O_2). Dissolved oxygen concentration in the environment depended on temperature and was measured with an oxygen sensor (Hach-Lange). The free radical production was evaluated with two specific probes:

- $O_2^{\circ-}$ production was evaluated by reduction of nitroblue tetrazolium NBT (0.5 mM) into formazan, measured by absorbance of formazan at 530 nm [4].
- OH° formation was evaluated by hydroxylation of terephthalate (1 mM) into hydroxy-terephtalate, measured by fluorescence spectroscopy (λex = 320 nm and λem = 420 nm) [5].

The kinetics were measured throughout 30 minutes using either NBT or terephthalate to avoid interaction between the probes. Different temperatures were tested from 4 to 75 ° C. All trials were repeated 4 times.

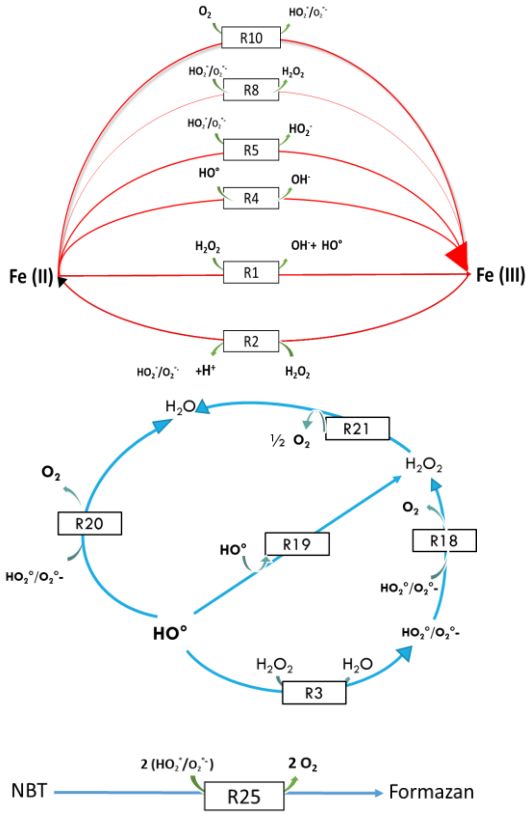


Figure 1: Chemical reactions in Fenton chemistry, free radicals scavenging, and NBT reduction.

Mathematical model

The stoichio-kinetic mathematical model [3] takes into account all the reactions in Figure 1 (possible ferryl iron formation was neglected). It consists of a set of Ordinary Derivative Equations (ODE). Each equation gives the rate of production/consumption of one chemical compound due to interaction of all the reactions involved. For example, the equation relative to H_2O_2 takes into account reactions R1, R2, R3, R18, R19 and R21 in Figure 1. The model parameters are the reaction rate constants and the activation energy of the limiting reactions (R1, R2, R10 and R21). Most of these parameters were taken from literature and from a previous work [6]. The hydroperoxyl ($HO_2°^-$) radical and its conjugated base, $O_2°^-$, were considered as unique chemical specie. Thus, apparent rate constants of reactions, in which they appear as reactant, were deduced from the actual rate constants and their relative concentrations. These latter were deduced from pH according to the predominance diagram (pKa = 4.8). The ODE system was solved with the solver "ODE15s", provided by the Matlab® software, which can cope with stiff systems. This was mandatory since the rate constants of the reactions involved in Fenton's chemistry vary over several orders of magnitude.

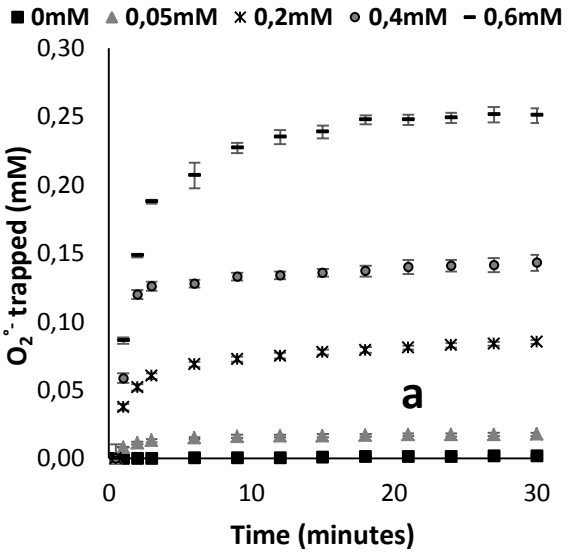
For a given set of initial concentrations of the compounds, simulation calculations provided the concentration kinetics of all compounds. Unknown parameters like a rate constant and its variation with temperature and pH can be fitted from the comparison between simulated and experimental kinetics. Here we focus on the activation energy of reaction R10 that is the first initiating reaction when no hydrogen peroxide is initially present.

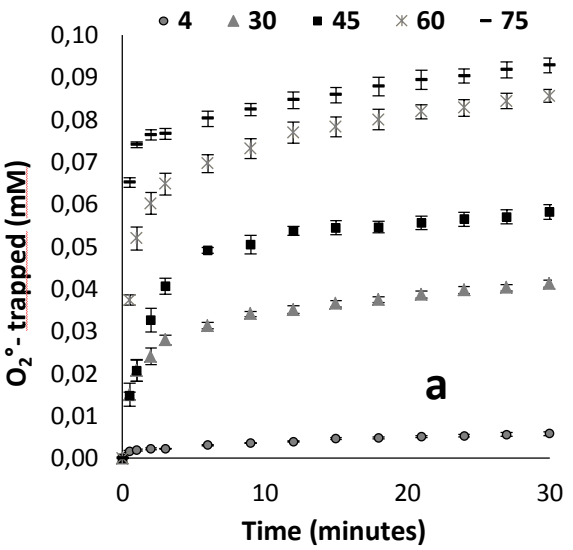
III. RESULTS AND DISCUSSION

The two probes proved to be stable at the temperatures tested, as well as in the presence of oxidants. In a first series of experiments, we studied the effect of ferrous iron concentrations at 60°C without H_2O_2. Figure 2 shows how much the amount of radicals reacting with probes increased with increasing concentrations of iron. The level of detected O_2° was significantly higher than that of OH°. The amount of $O_2^{\circ-}$ radicals increased proportionally to iron concentration. Such proportional increase was not observed for the hydroxyl radical OH°.

In a second series of experiments, iron concentration was set at 0.2 mM. The production of both free radicals increased with increasing temperatures (Figure 3) but differently. For example, at 4 °C, $O_2^{\circ-}$ production was very low while OH° was already high. Increasing the temperature from 4 to 70 °C promoted a 40 times increase in $O_2^{\circ-}$ production and just a 2 times increase in OH° production.

Addition of hydrogen peroxide in the mixture favoured the formation of OH° at the expense of superoxide radicals $O_2^{\circ-}$ (results not shown). With only iron added and in presence of dissolved oxygen (estimated here at 0.25 mM), superoxide radicals were formed according to the reaction R10 (Figure 1). This could explain the high content of $O_2^{\circ-}$ detected with NBT.





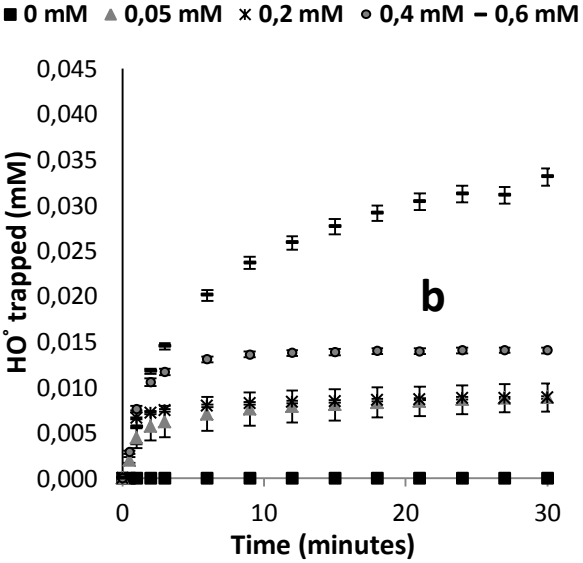


Figure 2: Effect of iron concentration on the production kinetics of the free radicals (temperature 60°C and no H_2O_2); **a** superoxide and **b** hydroxyl.

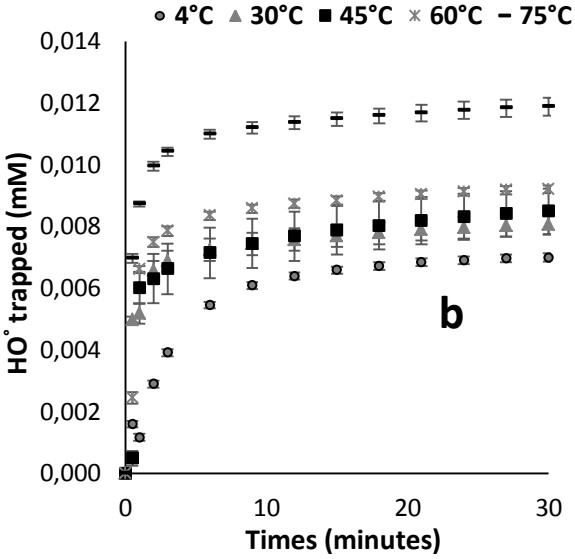


Figure 3: Effect of temperature on the production kinetics of the free radicals (Fe 0.2 mM, no H_2O_2); **a** superoxide and **b** hydroxyl.

Nevertheless, an important part of superoxide radicals may also disproportionate to produce hydrogen peroxide (reaction R18, Figure1) and ferrous iron can then react with hydrogen peroxide to form hydroxyl radicals (reaction R1, Figure 1). As iron reactivity with hydrogen peroxide is higher than with oxygen, a dramatic decrease in detected $O_2^{\circ-}$ was logically observed. In fact the situation is much more complex since all the chemical reactions in Figure 1 were interactive and it is helpful to resort to mathematical modelling.

To analyse $O_2^{\circ-}$ production by reaction R10 from the formazan production kinetics, it was first needed to assess the rate constant of reaction R25 (k_{25}). Oritani *et al.* [7] assessed a value of 10^4 M^{-1} s^{-1} from an analytical treatment of measured formazan production kinetics at ambient temperature for 25 ms. This rate constant value appeared extremely low and k_{25} was estimated again using our numerical model. Figure 4 shows that the calculations agree well with Oritani's measurements when k_{25} is equal to 1.2×10^8 M^{-1} s^{-1} (Figure 4).

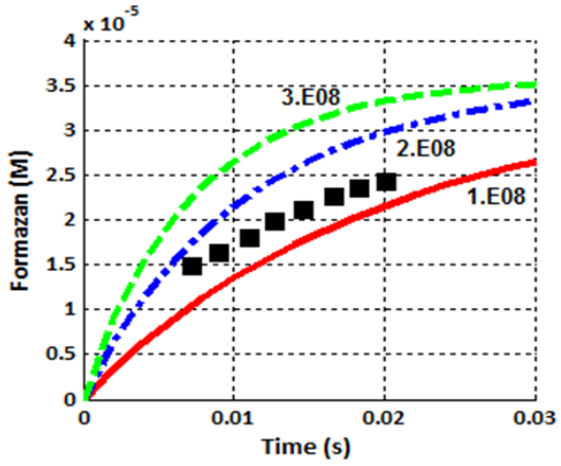


Figure 4: Kinetics of formazan production [7] after mixing 0.7 mM $O_2^{\cdot-}$ in DMSO solutions and 3.6 10^{-5} M water-soluble tetrazolium at ambient temperature in aqueous buffer solution (pH 9.2); Calculations (curves) and measurements (symbols)

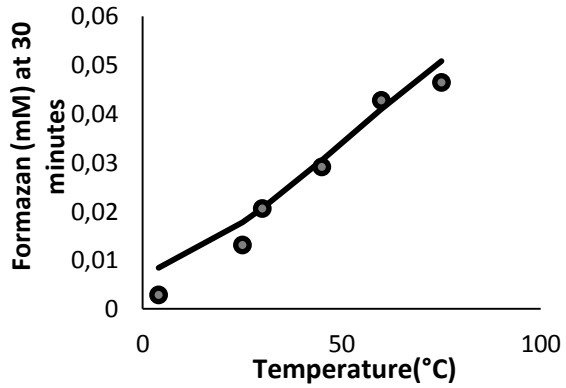


Figure 5: Comparison of calculated (curve) and measured (symbols) concentrations of formazan at 30 minutes *versus* temperature.

Figure 5 illustrates the use of the mathematical model to estimate unknown parameters. Here the experimental basis was the amount of formazan measured at 30 min. in trials with no H_2O_2, Fe 0.2 M and O_2 at saturated concentration. At 25°C, the reference temperature, the calculated value agreed with the experiment using the previous model [6] with k_{10} equal to 1500 M^{-1} s^{-1} Then the activation energy of the reaction R10 was adjusted to minimized the sum of squared differences between measurements in Figure 5 and calculated values; this energy is equal to 55 kJ/mol. The predicted levels of formazan at 30 min. agreed with the measurements but the calculated kinetics were steeper than those in figures 2 and 3. This might be due to iron complexation with phosphate or to ferric ions precipitation. Thus the present model should be refined by using a coefficient to modulate the actual amount of iron that can react with oxidants.

CONCLUSION

This work demonstrates the usefulness of using in parallel simplified experimental models and a stoichio-kinetic mathematical model to investigate free radical chemistry in meat. It showed the relative contribution of temperature and some oxidants but further studies are needed to consider haeminic iron. A data-base of rate constants and activation energies is under development to be able to predict lipid and protein oxidation during meat storage and cooking.

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REFERENCES

1. Haak, L., Raes, K.. & De Smet, S. (2006). Effect of dietary antioxidant and fatty acid supply on the oxidative stability of fresh and cooked pork. Meat Science 74: 476-486.
2. Gatellier, P. & Santé-Lhoutellier, V. (2009). Digestion study of proteins from cooked meat using an enzymatic microreactor. Meat Science 81: 405-409.
3. Van Boeckel, M.A.J.S. (2008). Kinetic Modelling of Reactions in Foods. Boca Raton: CRC press.
4. Auclair, C. & Voisin, E. (1985) Handbook of Methods for Oxygen Radical Research. Boca Raton: CRC Press.
5. Sarran, M. & Summer, K.H. (1999). Assaying for hydroxyl radicals: hydroxylated terephthalate is a superior fluorescence marker than hydroxylated benzoate. Free Radical Research 31: 429-436.
6. Promeyrat, A. (2013). PhD thesis, Université de Clermont-Ferrand, France.
7. Oritani, T., Fukuhara, N., Okajima, T., Kitamura, F. & Ohsaka, T. (2004). Electrochemical and spectroscopic studies on electron-transfer reaction between novel water-soluble tetrazolium salts and

a superoxide ion. Inorganica Chimica Acta 357(2):
436-442.