# Kinetics of sodium nitrite degradation, nitrate and nitric oxid-haem pigment development during Mortadella type sausage cooking

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*Abstract*— **This study was carried out to analyze the behavior of nitrite in the manufacturing process of low-sodium nitrite cooked meat.**

**The aim of this work was to investigate the relationship between the sodium nitrite ingoing amount and cooking temperature in order to find the best combination for a good development of the colour and low associated residues in Mortadella Bologna type sausage.**

**Mortadella Bologna type sausages were manufactured adding four levels of sodium nitrite (from 40 to 150 mg/Kg) and using five different cooking temperatures (from 55° to 72°C). The analysis was focused on the first phase of the cooking process, where the nitrosohaemocrome is about fully developed, while the nitrite degradation is just started.**

**Within a 90 minute interval, on a frequency of 15 minutes, a 3 mm thick-outer circle was taken from all samples, it was cooled, grounded and finally the analyses of nitrite, nitrate and nitric oxide–haem pigments were performed. The kinetic parameters of nitrite dismutation/reduction reactions and nitric oxide-haem pigment development were calculated.**

**The sodium nitrite dismutation reaction (Ea=33Kj/mol) was less sensitive to the temperature increase of the corresponding reduction reaction (Ea=53Kj/mol); the dismutation reaction kinetic constant ($K_v^d$=1-1.5x$10^{-3}$min$^{-1}$) increased less than the reduction reaction corresponding one ($K_v^r$=0.3-3x$10^{-3}$min$^{-1}$) raising the temperature from 55°C to 72°C. At 65°C the latter reaction prevailed. The nitric oxide pigment development was the fastest reaction ($K_v^n$=8-10x$10^{-3}$ mol$^{0.5}$L$^{-0.5}$ min$^{-1}$) and it was mostly independent from temperature.**

**The maximum nitrosation and the lowest residues were achieved with 70 mg/Kg of sodium nitrite and a working temperature of 65°C.**

*Keywords*— **Mortadella, nitrite, kinetic**

## I. INTRODUCTION

Mortadella is a chemically complex system, composed of two immiscible phases: a lipid phase which accounts for about 30% of the product and a fraction in which muscle protein filaments are dispersed in aqueous phase containing proteins and minerals.

The reactions that occur in aqueous phase, probably proceed in different ways compared with in vitro reactions, because of the phenomena that take place during the cooking process: interactions between many molecules, the combining effects of temperature and the migration of fat and water out of the system [1-3].

For this reason the study of kinetics of reactions performed during a real process of cooking, takes a different value from a study done on a model system. The sodium nitrite, a common mortadella additive, is involved in some reactions stabilizing color, influencing flavor and giving microbiological safety [4-8].

The aim of this work was to investigate the relationship between the sodium nitrite ingoing amount and the cooking temperature, in order to find the best combination for a good development of the colour, safety of the product and low associated residues in Mortadella Bologna type sausage.

## II. MATERIALS AND METHODS

### A. Experimental design

Mortadella Bologna type sausages were manufactured adding NaCl (2%), ascorbic acid (0.05%) and four levels of sodium nitrite (40, 70, 100 and 150 mg/Kg) and using five different cooking temperatures (55°, 60°, 65°, 70° and 72°C).

Within a 90 minute interval, on a frequency of 15 minutes, a 3 mm thick-outer circle was taken from all samples, it was cooled, grounded and finally analyzed.

*B. Kinetic model*

Our study suggests and examines the following reactions:

$$3\ NO_2^- + 2H^+ = 2\ NO + NO_3^- + 2H_2O \quad (1)$$
$$Red + NO_2^- = NO + Ox \quad (2)$$
$$Pig + NO = NOP \quad (3)$$
$$Subs + NO = decompos. \quad (4)$$

The disappearance of nitrite ($NO_2^-$) follows two paths: the dismutation to nitric oxide (NO) and nitrate (reaction 1), or reduction (reaction 2). Nitric oxide reacts in turn with both the meat pigment (reaction 3), and with many other substrates, including organic, exerting a bacteriostatic action [9] (reaction 4). The experimental data concernig concentration of nitrate ($NO_3^-$), nitric oxide haeme (NOP) and residue of nitrite, are acquired from samples taken at different times throughout cooking process. The concentration of NO, due to the difficulty of monitoring this molecule, was evaluated from disappeared nitrite and from development of nitrate and NOP, depending on their stoichiometric ratio.

*C. Analytical methods*

*Determination of nitrite and nitrate concentrations*

The nitrite and nitrate concentrations were measured in all samples collected by ion suppression chromatography using the modified method SSICA [10].

*Nitric oxide-haem pigment measurement*

The nitric oxide-haem pigments were determined by spectrophotometry following the method described by Hornsey [11]. The concentration was expressed as milligrams of NO-haematin per 1Kg of sample (mg/Kg).

## III. RESULTS

The global disappearance of nitrite roughly occurs according to a first-order kinetics as well as the dismutation reaction and formation of nitrate. The table 1 shows the rate constants (Kr) of the four reactions at different temperatures.

Table 1. Kr of nitrite degradation, developing of the nitrous haeme and nitric oxide, and free nitric oxide output.

| Temperature (°C) | Nitrite dismutation ($min^{-1}$) | Nitrite decreasing ($min^{-1}$) | Develop. of NO-haematin ($mol^{0.5}\ L^{-0.5}\ min^{-1}$) | Free Nitric oxide output ($min^{-1}$) |
|---|---|---|---|---|
| 55 | $0.9 \times 10^{-3}$ | $1.8\times10^{-3}$ | $7.2 \times 10^{-3}$ | $2.3\times 10^{-3}$ |
| 60 | $0.8 \times 10^{-3}$ | $1.4\times10^{-3}$ | $7.6 \times 10^{-3}$ | $5.5 \times 10^{-3}$ |
| 65 | $1.2 \times 10^{-3}$ | $3.7\times10^{-3}$ | $9.9 \times 10^{-3}$ | $18 \times 10^{-3}$ |
| 70 | $1.5 \times 10^{-3}$ | $4.2\times10^{-3}$ | $11.8 \times 10^{-3}$ | $11.1 \times 10^{-3}$ |
| 72 | $1.6 \times 10^{-3}$ | $3.9\times10^{-3}$ | $10 \times 10^{-3}$ | $1.7 \times 10^{-3}$ |

The nitrite consumption is promoted by increasing temperature while dismutation reaction is less affected by temperature just as by initial concentration of nitrite. Instead, rate of reduction reaction increases both with the temperature and the ingoing amount of nitrite. (table 2)

Table 2. Activation energy (Ea) calculated from kinetic data

| Reaction | Ea (KJ/mole) |
|---|---|
| Nitrite dismutation | 33.4 |
| Nitrite decreasing | 53.1 |
| Developing of nitric oxide heme | 23.9 |

At low temperatures, or with low nitrite ingoing, the developing of nitric oxide and nitrate (according to the reaction 1) is the main effect. When the temperature increases and the addition of nitrite enhances, the main reaction becomes the direct reduction that turns out to be central to the formation of nitric oxide, enough for color development and for microbiological safety. The formation of nitric oxide pigment requires very low concentrations of nitric oxide, in fact the sample obtained at 55 ° C and with only 40 mg/Kg of added nitrite, already had a pink color, even if the pigment formed was slightly lower than that obtained at higher temperatures. Figure 1 shows the trend of nitric oxide haematin developed at different ingoing of nitrite at 65°C. An amount ranging from 70 and 100 mg/Kg of nitrite is enough to provide almost 70 mg/Kg of NOP and, in addition, more than 50 mg/Kg of nitric oxide (figure 2).

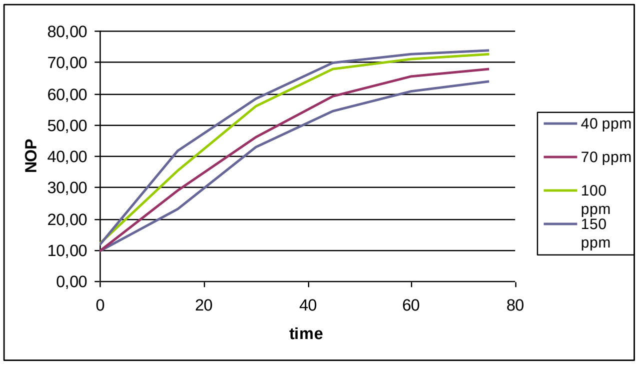


Fig. 1 NO-haematin concentration (mg/Kg) against cooking time at 65°C at different nitrite ingoing amount.

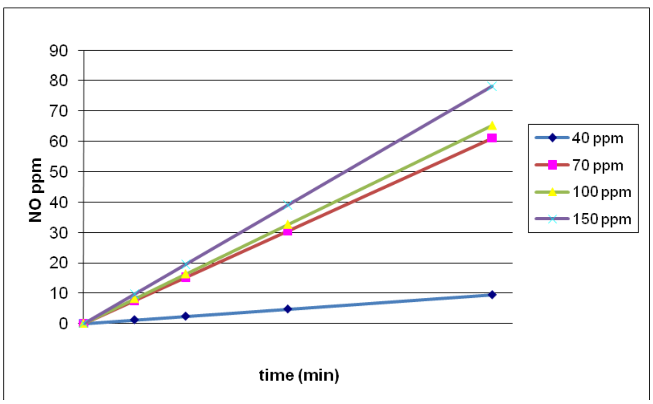


Fig. 2 Evaluation of nitric oxide formation (mg/Kg) against cooking time at 65°C at different nitrite ingoing amount

The conversion rate of the pigments increases up to 70 °C, as shown in Figure 3.

Because NO-haematin follows a half-order kinetics with respect to the concentration of pigment, the reaction rate remains higher than other concurrent reactions to the same concentration of the reagent, as can be seen from its concave trend in figure 4.

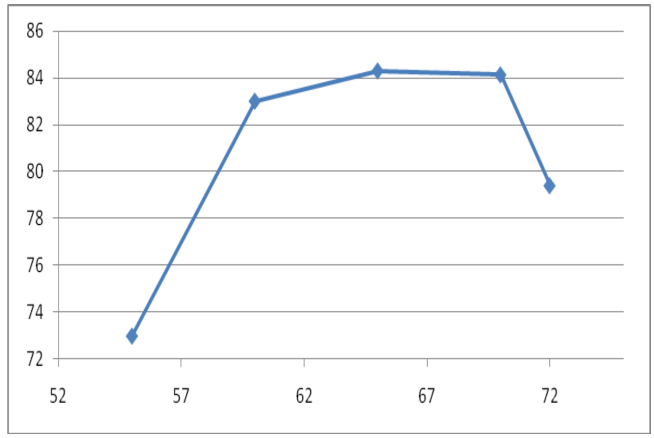


Fig. 3. Conversion percentage of the native pigment to NO-haematin pigment after 90 minutes at different temperatures.

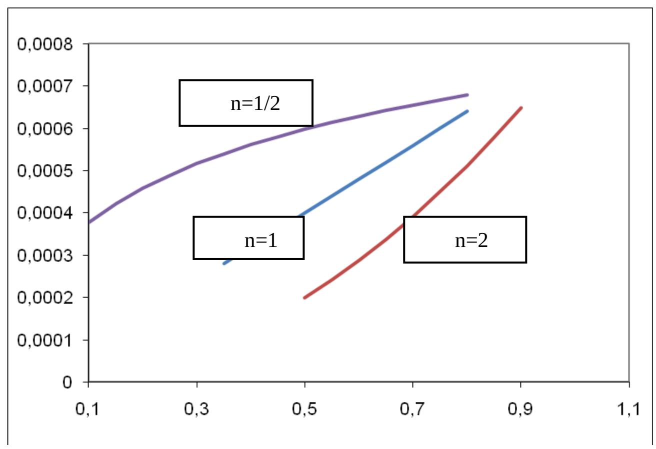


Fig. 4. Reaction rate versus the concentration of the limiting reagent, for different order of reaction.

## IV. DISCUSSION

We can therefore state that the pigments nitrosation reaction is the favorite one, even for the low activation energy, and consumes the first formed nitric oxide. Already at 55 ° the NO-haematin is formed with a sufficient conversion after one hour with at least 70 mg/Kg ingoing amount of nitrite. In this case nitric oxide begins to develop at high concentration only above 100 mg/Kg of nitrite ingoing. Below this concentration the dismutation prevails and thus nitric oxide is produced at low concentrations. When adding more than 100 mg/Kg of nitrite, the nitric oxide produced in excess of consumption due to heme reaction, interacts even more with biological systems of microorganisms inhibiting their growth and carrying out its protective action.

The maximum production of nitric oxide heme occurs at 65 ° between 70 and 100 mg/Kg of nitrite added. Under these conditions about 16 mg/Kg of nitric oxide per hour are generate; since it takes 2 or 3 hours to consume 1 or 1.44 mmol of nitrite ingoing (corresponding to 100 and 150 mg/Kg of sodium nitrite), 32 - 48 mg/Kg of nitric oxide are generated during heating to 65 ° C. These are the minimum conditions to obtain a pink color and safety product, with reduced nitrate content. The reaction fo reduction of nitrite to nitric oxide involves reducing molecules present in meat such as cysteine or additives as ascorbate.

The rate of reduction (reaction 2) is more dependent from temperature. The production of nitric oxide has a 10-fold increase of its rate (0.003 mmol / min to 0.03 mmol / minute) when temperature is enhanced from 55 ° to 72 °. This means that the added nitrite is consumed in about 6 hours at 55 ° producing less than 30 mg/Kg, while at 72 ° the nitrosylation proceeds in just under two hours and about 90 mg/Kg of nitric oxide would be produced.
The temperature of 55 ° is therefore not sufficient for the safety of the product while at 72 ° the nitric oxide exceeds the functional amount. The formation of nitrate has not been considered in the study. It proceeds over time if the conditions are favorable and always if the residual nitrite is sufficient.

## V. CONCLUSION

During the manufacturing of mortadella, for effect of additives, some compounds which can be hazardous to the health are produced, but also useful products to the formation of color and to its microbiological safety are developed. To this end, the addition of 70 mg/Kg sodium nitrite and a heat treatment to achieve an endpoint temperature of 65 ° C are sufficient conditions for developing pink color of the product and to achieve a concentration of nitrite that could be effective to inhibit the growth of pathogens joint with other conditions. The reaction of nitrite reduction is temperature dependent and below 65 ° the formation of nitric oxide is promoted. Even at these temperatures nitrosylation of the pigment takes place efficiently, because it is a fast stage of the process and proceeds as well at low temperatures.

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