Kinetics of nitrite evaluated in a meat product

G. Barbieri *, M. Bergamaschi, Ge. Barbieri, M. Franceschini

Stazione Sperimentale per l'Industria delle Conserve Alimentari, 43100 Parma, Italy

ARTICLE INFO

ABSTRACT

The evaluation of the efficiency with which the reactions involving nitrite proceed in mortadella and of the effect exercised on their kinetics by some variables (ingoing amount of sodium nitrite and temperature) is the purpose of this work. Kinetics parameters were calculated at each level of nitrite added (40, 70, 100 and 150 mg/kg) and at five temperature (55°, 60°, 65°, 70° and 72 °C). While the colour formation reaction is favoured by low activation energy, it becomes crucial to enable nitrite to proceed according to direct reduction thus preventing an increase in nitrate concentration as well as an excess of nitric oxide in the product. Kinetics data suggest that this scope is performed when the product achieves the temperature of 65 °C as fast as possible with an ingoing amount of sodium nitrite of 70 mg/kg.

1. Introduction

Sodium nitrite is an additive used widely in meat products. It is involved in a number of functions: 1) it controls the development of some pathogenic species (Duffy, Vanderlinde, & Grau, 1994; Glass, McDonnell, Rassel, & Zierke, 2007; Yetim, Kayacier, Kesmen, & Sagdic, 2006); 2) it develops the typical pink colour of cooked ham (Cornforth & Jayasingh, 2004; Lawrie, 1998); 3) it contributes to flavour formation (Guillard, Goubet, Salles, Le Quéré, & Vendeuvre, 1998); and 4) it exerts an antioxidant action against fats (Gatellier, Lessire, Hermier, Maaroufi, & Renerre, 2003; Han & Yamauchi, 2000). On the other hand, nitrite also plays a substantial role in formation of the carcinogenic N-nitrosamine in meat, especially under the process conditions applied in the meat processing industry (Juncher et al., 2000; Lijinsky, 1999; Ward et al., 2007).

Numerous studies have been carried out to replace nitrite in meat products (Dineen et al., 2000; Kawahara, Nakamura, Sakagami, & Suzuki, 2006; Pegg & Shahidi, 1997; Shahidi & Pegg, 1995; Sørheim et al., 2006; Viuda-Martos et al., 2009), but so far none of the alternatives found are as effective in colour formation or bacteriostatic action on pathogenic species such as listeria and clostridium (Lucke, 2008). Reducing the use of nitrites has also been the subject of studies (Hammer, 1998) by European legislators (Dir 2006/52/CE; Dec UE 2010/561).

Mortadella is a product made with finely ground pork meat (shoulders and trimmings from other cuts) combined with diced pork fat taken from the throat or belly, which is stuffed into natural casing or, more frequently, synthetic casing. This product requires a long cooking time in a dry air oven, during which numerous chemical reactions can take place between the various meat components and additives (Cornforth & Jayasingh, 2004; Fox, 1966; Sebranek & Fox, 1985). The reactions involving nitrite can be classified, for the sake of simplicity, as concurrent reactions that consume it (dismutation and reduction), and produce, among other compounds, nitric oxide (NO). Nitric oxide, in turn, also reacts in various ways, concurrent with one another. At the pH of meat, generally 5.65 up to 5.85, nitrite is mostly present in the dissociated form, nevertheless, the reactivity of undissociated forms leads to the formation of several intermediates, which are unstable and therefore difficult to determine, especially in a real system subjected to thermal treatment. Nitrite consumption can be due to the action of reducing substances endogenous to the meat, e.g. sulphur-containing amino acids (cysteine), or added ones such as ascorbate. In addition, a nitrite dismutation reaction also results in nitrate formation. The consumption of NO occurs by a reaction with both the denatured pigment of meat and with some substrates present in the mixture, such as the biochemical cellular systems of microorganisms, which prevents their growth and preserves the meat product from a microbiological point of view (Reddy, Lancaster, & Cornforth, 1983). Therefore, the amount of NO developed is an important parameter when evaluating the effect of added nitrite on the microbiological shelf-life of the product, with the aim of minimizing excess. In addition to reduce the nitrite addition, it is also important to keep the residual of nitrate and nitrite residues as low as possible. So far, the kinetics of nitrite has been studied only on model systems (Fox et al., 1994; Geileskey et al., 1998).

The purpose of this work was to study the kinetics of nitrite decreasing by following the evolution of analytically measurable chemical species (NO_3, NO_2, total pigments, and nitric oxide pigments) and calculate the content of NO through stoichiometry of the reactions considered during the production of mortadella in an industrial plant. In addition, we evaluated the efficiency with which, in a real system, important

---

* Corresponding author.
 E-mail address: giampiero.barbieri@ssica.it (G. Barbieri).

and technologically useful reactions proceeded and determined the effects of some variables (ingoing amount of sodium nitrite and temperature).

2. Materials and methods

The evolution of the nitrite/nitrate/nitric oxide/nitric oxide haem complex was studied experimentally on mortadella mixtures prepared in pilot plant, under stationary temperature conditions obtained in a dry air oven normally used for industrial production of mortadella. The raw mixture consisted of about 62% of moisture, 17% of proteins and 20% of fat.

The evolution of the system was followed by taking a sample on a superficial layer when the temperature was balanced with the temperature of the oven.

2.1. Manufacturing of mortadella

The oven was set to five different cooking temperatures: 55°, 60°, 65°, 70°, and 72 °C. In these stationary conditions, we cooked mortadella mixtures with a variable content of added sodium nitrite (40, 70, 100, and 150 mg/kg), while the percentage of sodium chloride (2%) and sodium ascorbate (0.05%) was kept constant. Superficial samples were collected at set times according to the experimental plan described in Fig. 1. The samples were immediately cooled down to stop the development of further reactions. Each sample was ground: one aliquot was used for extracting and determining nitric oxide pigments, while the other aliquot was analyzed for nitrite and nitrate.

2.2. Nitrite and nitrate determination

Ion suppression chromatography using the SSICA modified method was used to measure nitrite and nitrate contents in all samples collected (Pizza et al., 2007).

2.3. Nitric oxide-haem pigments measurement

The total pigments and nitric oxide-haem pigments were determined by spectrophotometry following the method described by Hornsey (1956). The content was expressed as milligrams of haematin and NO-haematin per 1 kg of sample (mg/kg).
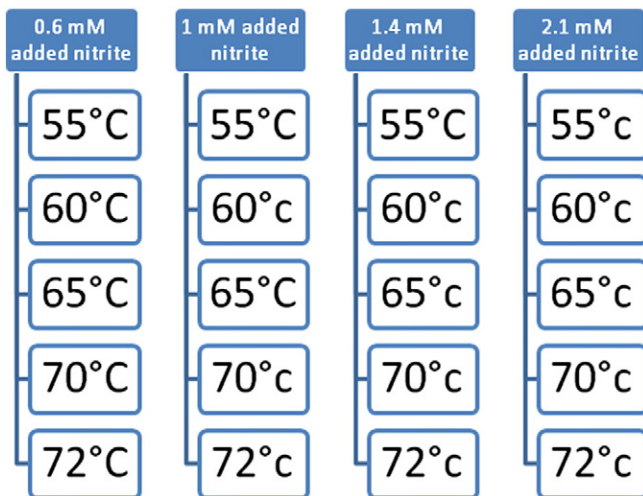

Fig. 1. Experimental design.

2.4. Kinetics study

Overall, the reactions of the chemical species involved were expected to be as follows, as mentioned in the literature (Honikel, 2008; Sebranek & Fox, 1985):

$$3NO_2^- + 2H^+ \rightarrow 2NO + NO_3^- + H_2O \tag{1}$$

$$NO_2^- + Red \rightarrow NO + Ox \tag{2}$$

$$XNO + Pig \rightarrow NOP \tag{3}$$

$$XNO + Subs \rightarrow \tag{4}$$

where: Red and Ox are the reduced and oxidized form of the same molecule, Pig is total pigments an NOP is nitric-oxide pigments.

Although some of these are actually multiple stage reactions, this study looked at the overall reaction. It was not possible to use sophisticated sampling methods in this "real product" system; and measurements were limited to the most stable chemical species: nitrite, nitrate, total pigments, and nitric oxide pigments, while the extremely unstable NO species was determined by stoichiometry of the above reactions through concentrations of the other chemical species. We measured the overall disappearance rate of the nitrite added, including its participation in two concurrent reactions: dismutation (reaction 1) and direct reduction (reaction 2).

From the content values (obtained from two replications) at different times, we calculated the reaction rate for each experiment.

Data were processed using SPSS 14.1 software.

3. Results and discussion

The logarithm of residual nitrite content, consumed overall by reactions 1 and 2, showed a linear pattern over time:

$$\ln[NO_2^-] = K_{TOT}t; \text{ and the reaction rate is } r_{TOT} = \left[\frac{d[NO_2^-]}{dt}\right] TOT$$
$$= K_{TOT}[NO_2^-]^1$$

where r_{TOT} overall rate of nitrite decreasing,
K_{TOT} constant rate of nitrite decreasing.

The content of nitrite involved in the dismutation (reaction 1), evaluated from the experimental determination of nitrate content, also decreased logarithmically as the reaction progressed. Therefore, the kinetics of reaction 1 and of the overall reaction of nitrite disappearance could be assumed to approximate first-order with respect to NO_2^-, although the dismutation reaction involves three nitrite molecules. Since reducer species (such ascorbate) are not a limiting factor, we could also assume that nitrite reduction (reaction 2) followed first-order kinetics.

The overall rate with which nitrite is consumed is given by the sum of the rates of the single dismutation and reduction reactions. Therefore, the rate with which nitrite is consumed by reduction (reaction 2) is calculated as the difference: $r_r = r_{TOT} - r_d$ where r_r is rate of reaction 2) and r_d is the rate of reaction 1).

The kinetics equations corresponding to the dismutation (d) and reduction (r) reactions are as follows:

$$r_d = K_d[NO_2^-]^n = -1/3\left[\frac{d[NO_2^-]}{dt}\right]_d = \frac{d[NO_3^-]}{dt} = \frac{1}{2}\left[\frac{d[NO]}{dt}\right]_d$$