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Diffusion of nitrate and water in pork meat: Effect of the direction of the meat fiber

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ABSTRACT

The effect of the direction of the meat fiber on the diffusion of sodium nitrate and water in *Semimembranosus* pork muscle during curing was studied at different temperatures. Nitrate and water diffusion were modelled based on Fick's second law. The nitrate diffusion coefficients ranged from $0.007 \cdot 10^{-10}$ to $0.034 \cdot 10^{-10}$ m^2/s (parallel) and $0.89 \cdot 10^{-10}$ to $1.41 \cdot 10^{-10}$ m^2/s (perpendicular), while for water the values ranged from $9.87 \cdot 10^{-9}$ to $12.46 \cdot 10^{-9}$ m^2/s (parallel) and $5.22 \cdot 10^{-10}$ to $9.29 \cdot 10^{-10}$ m^2/s (perpendicular). In every case, these values increased as the temperature rose. The activation energy for water diffusion perpendicular to the meat fiber (31.86 kJ/mol) was greater than when the diffusion was parallel (15.06 kJ/mol). The opposite was observed for nitrate diffusion (96.44 kJ/mol when parallel vs. 24.71 kJ/mol when perpendicular), which implies that nitrate needs more energy for parallel diffusion and, consequently, curing is slower in that direction.

1. Introduction

Curing agents (nitrate and nitrite) are essential ingredients for cured meats. They play an important role in both color and flavor development and are also antioxidant (Honikel, 2008). Furthermore, nitrite exerts a significant antimicrobial effect in dry-cured products related to the inhibition of the growth of several pathogens (Hospital et al., 2012), while nitrate is effective as a curing agent when it is reduced to nitrite by means of the meat microbial flora with nitratereductase activity (Toldrá, 2007). Nitrite is very reactive as a curing agent, thus it is quickly depleted in cured meats, specifically in the surface regions where it is formed. Consequently, the amount of nitrite penetrating into the center of the product is reduced (Arnau et al., 2003), and its preservative effect inside the product is lost. The use of nitrate as a slow source of nitrite is a way to introduce nitrite into the inner parts of cured meats, especially for large pieces aged for long periods, where there exists a greater risk of microbial growth (Toldrá, 2007).

Apart from the effects of nitrate and nitrite on meat quality, the therapeutic potential of these salts for cerebrovascular accidents, myocardial infarction or hypertension has been demonstrated in recent studies (Rocha et al., 2011; Butler, 2015). Nevertheless, nitrite involves the potential formation of nitrosamines through a reaction with secondary amines, which have teratogenic, mutagenic and carcinogenic effects (Viera et al., 2006). In fact, the World Health Organization (WHO, 2015) categorized processed meat as Group 1, carcinogenic to humans, and red meat as Group 2A, probably carcinogenic to humans.

Potassium and sodium nitrate and nitrite are currently restricted in the EU by Regulation no. 1129/2011 (EC, 2011). Nevertheless, the claims of the WHO point out to a more strict regulations aimed to reducing the amount of curing salts. However, it must be borne in mind that reducing the nitrate added to meat could affect the quality and safety of the cured products (Toldrá, 2007). For that reason, it becomes necessary to assess the effect of reducing the amount of added nitrate in meat products.

The reactions and transformations of nitrates and/or nitrites in meat depend on their diffusion rate (Arnau et al., 2003). The study of nitrate diffusion in meat is essential for the purposes of monitoring the curing process (Graiver et al., 2006). Effective diffusivity can be calculated by means of diffusion models and can be used for this purpose (Zhang et al., 2011). Moreover, it is well known that the

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diffusion coefficient is temperature dependent (Gou et al., 2003; Pinotti et al., 2002).

In the literature, studies about diffusion of water and sodium chloride in fish and meat products can be found (e.g. Andreetta-Gorelkina et al., 2016; Bampi et al., 2016). The distribution of nitrate in different muscles during the ageing of ham, dry-cured with KNO_3, has also been studied (Arnau et al., 1995). Nevertheless, to our knowledge, neither the diffusive behaviour of sodium nitrate inside the meat nor the distribution of nitrate inside meat samples that have been cured with $NaNO_3$ saturated brine, have been published. The distribution of nitrate is important in order to find out its penetration velocity in meat (Bertram et al., 2005). Furthermore, studying the kinetics of nitrate diffusion in meat products can help to maintain an appropriate nitrite concentration during the subsequent stages of processing.

Likewise, the direction of the muscle fiber is another parameter that affects the diffusion coefficient. Some studies show the influence of the orientation of the fiber on the diffusion of sodium chloride and water during meat curing (McDonnell et al., 2013; Gou et al., 2002; Zhang et al., 2011). The determination of the diffusion coefficient as a function of the direction of the muscle fiber can contribute substantially to an understanding of nitrate mobility in meat.

Based on the above-mentioned aspects, and considering the interest there exists in knowing how isolated curing salts behave, the objective of this study was to gain an insight into the effect of the direction of the meat fiber on the diffusion kinetics of sodium nitrate and water in the *Semimembranosus* muscle of pork leg during its immersion curing in a brine saturated with $NaNO_3$ at different temperatures.

2. Materials and methods

2.1. Raw material

Fourteen pork legs (average weight, 9.6 ± 1.2 kg; pH 45 h *post mortem* > 6.0 and pH 24 h *post mortem* = 5.9 ± 0.1, measured in *Semimembranosus* muscle), were selected from a local slaughterhouse. All muscles were obtained the day before the experiments from different animals. The meat storage conditions and the way in which samples were obtained have been previously described in Gómez et al. (2015). The legs were divided into two groups: a first group of 6 legs, from which 84 cylinders (8.4 cm in height and 2.4 cm in diameter) were obtained for experiment I; and a second group of 8 legs, from which 96 cylinders (same size than for experiment I) were obtained for experiment II (88 cylinders for curing kinetics and 8 cylinders for determining the equilibrium concentration of nitrates). Experiments I and II are described below.

2.2. Experimental methods

2.2.1. Experiment I

Experiment I was designed to study water and $NaNO_3$ diffusion parallel to meat fibers (water and nitrate transport in axial direction) at 2, 7 and 12 °C and 95 ± 1.5% relative humidity. The experimental procedure is similar to the one described in Gómez et al. (2015) for nitrite. The cylinders were weighed and their side face was subsequently covered with a PVC film to prevent moisture loss. Each cylinder was hung from one of its bases and the other one was in contact with a brine saturated with $NaNO_3$.

2.2.2. Experiment II

To study water and $NaNO_3$ diffusion perpendicular to meat fibers, cylinders were weighed and immersed in a brine saturated with sodium nitrate ($NaNO_3$). Since the length of the cylinders is around 4 times the diameter, an infinite cylinder geometry can be considered, thus diffusion takes place in radial direction, that is, perpendicular to the fibers. The concentration of sodium nitrate in the experiment conditions was between 42.2% (0 °C) and 46.2% (12 °C) (Fig. 1) and the volume solution to meat weight ratio was approximately 5:1 (v/w). The saturated brine and the cylinders were randomly placed into curing chambers at 0, 4, 8 and 12 °C (eleven cylinders per chamber, two chambers at each temperature) with 95± 1.5% relative humidity. The curing chambers were also placed inside a chamber with controlled temperature and relative humidity. The measurement, monitoring and control of temperature and relative humidity inside the curing chambers were performed as in experiment I (see Gómez et al., 2015).

The curing process was carried out for 5 days. Every 12 h, one of the cylinders was removed from the brine and, by using a bore, two sections were obtained: an internal one of 1.2 cm diameter and an external one (Fig. 1).

2.3. Analytical techniques

The pH was measured using a Mattäus model pH-STAR CPU lab pH-meter (Pötmes, Germany). The initial water content was determined by the AOAC methodology (AOAC, 1997). In experiment I, the evolution of the mean average moisture content of each cylinder over time was determined through the weight difference, based on the initial moisture content. For experiment II, the water content was determined in quadruplicate for each cylinder section (AOAC, 1997).

The cylinders in experiment I were cut into 4 slices (A to D) and the nitrate content of each slice was determined (Gómez et al., 2015). For the cylinders of experiment II, the nitrate content was determined for both sections, the external and the internal one.

For the purposes of determining the nitrates, 5 g of meat tissue, previously triturated using Mini-mixer equipment (Ufesa BP4530), and 200 ml of water from a MilliQ plus system (Millipore, Billerica, MA, USA), were placed in a 300 ml volumetric flask. The flask containing the mixture was placed in a bath at 100 °C and heated for 10 min. The suspension was homogenized for 10 min at 9000 rpm using an Ultra-turrax T25 (IKA Labortechnik, Janke & Kunkel GMBH & Co, Staufen, Germany). The homogenate was subsequently diluted with water (MilliQ plus system) and filtered (Waterman #1) to obtain the extract. The nitrate content of the extract was determined in triplicate by using the Method 4500-NO3 Nitrogen (Nitrate) (APHA et al., 1998). For that purpose, the

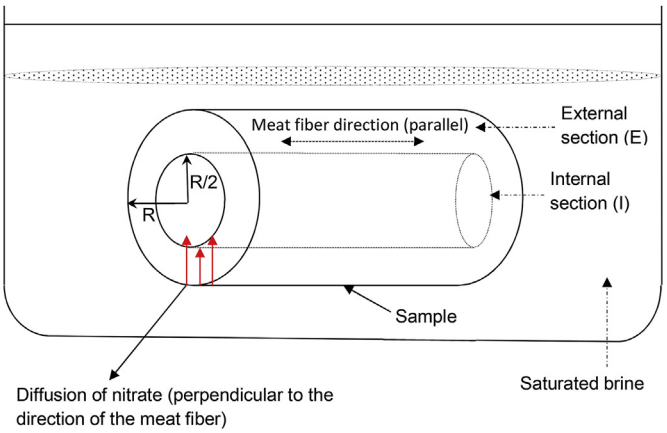

Fig. 1. Sections into which the meat cylinders were divided in order to analyze nitrate content. Nitrate diffusion perpendicular to the direction of the meat fiber.

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