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.

Keywords

Meat, curing, diffusion, direction of fiber, nitrate, water

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 (Commission Regulation (EC) No 1129/2011). 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 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 hours *post mortem* > 6.0 and pH 24 hours *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 ºC 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% (0ºC) and 46.2% (12ºC) (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 12ºC (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 hours, 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 100ºC 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-NO_3^- Nitrogen (Nitrate) (APHA, AWWA and WEF, 1998). For that purpose, the nitrate present in a portion of the extract was reduced to nitrite by means of a copperized cadmium column. The eluate of this reduced extract was used for nitrate plus nitrite determination, while the remaining unreduced portion was used for nitrite determination. In both the unreduced and reduced solutions, the colour red was developed by N-(1-Naphthyl)ethylenediamine and sulphanilamide addition, and the absorbance was measured at 538 nm (Gómez et al., 2015). Nitrate content was calculated from the difference between the two measurements.

The method was validated by injecting a known amount of $NaNO_3$ into small pieces of meat, and comparing those amounts with the values obtained following the extraction and determination procedure described above. The method was successfully validated (R^2 = 0.99).

2.4. Modelling

The mass transfer during meat curing in both experiments was modelled, based on the analytical solution of Fick's second law of diffusion.

2.4.1 Modelling mass transport parallel to the direction of the meat fiber

The modelling of the experimental values from experiment I for both water exit and nitrate penetration is described in Gómez et al. (2015). To determine the equilibrium concentration of nitrates, two cylinders were left in contact with the saturated brine at each temperature (0ºC, 4ºC, 8ºC and 12ºC) until constant concentration (30 days). The equilibrium moisture content of the samples was calculated from Peleg´s model (Peleg, 1988).

The estimation of the effective diffusivities was performed considering an optimization problem that was solved by SOLVER, a tool included in EXCEL™ (Microsoft), selecting a non-linear optimization method (Generalized Reduced Gradient). The effective diffusivity values of nitrate (D_{Ne}) and water (D_{we}) were calculated by minimizing the mean of the square differences between the concentrations measured in the experiment and those calculated by the model. The goodness of fit was evaluated by means of the explained variance (%var).

2.4.2 Modelling mass transport perpendicular to the direction of the meat fiber

We assumed one-dimensional transport perpendicular to the direction of the meat fiber (infinite cylinder geometry), negligible external resistance to mass transfer, solid homogeneous and isotropic, constant effective diffusivity and constant dimensions of the samples throughout the experiment.

Taking into account the initial and boundary conditions, the solution of the governing equation gave Equation (1):

$$\frac{C(r,t)-C_e}{C_0-C_e} = 2\sum_{n=1}^{\infty} \frac{e^{-D_e \lambda n^2 t}}{\lambda n R J_1(\lambda n R)} J_0(\lambda n r) \qquad (1)$$

being $\lambda nR/J_0(\lambda nR) = 0$

The average nitrate content for both the internal cylinder (I) and the external one (E) at a given time t, $\overline{C}_s$, was calculated by integrating Eq. (1) between 0 and R/2 (Eq. (2)), for section I, and between R/2 and R, for section E (Eq. (3)) (Fig. 1):

$$\frac{\overline{C}_{s\,(0-R/2)} - C_{se}}{C_{s0}-C_{se}} = 8\sum_{n=1}^{\infty} \frac{e^{-D_{Ne} \lambda n^2 t}}{(\lambda n R)^2 J_1(\lambda n R)} J_1(\lambda n \frac{R}{2}) \qquad (2)$$

$$\frac{\overline{C}_{s(R-R/2)} - C_{se}}{C_{s0} - C_{se}} = \frac{16}{3}\sum_{n=1}^{\infty} \frac{e^{-D_{Ne}\lambda n^2 t}}{(\lambda n R)^2 J_1(\lambda n R)} \left[J_1(\lambda n R) - J_1(\lambda n \frac{R}{2}) \right] \tag{3}$$

being $\lambda nR/J_0(\lambda nR) = 0$

The equilibrium concentration of nitrates was established by determining the nitrate content after the immersion of a cylinder in the brine for 7 days.

The equations for the calculation of the average moisture content in section I (Eq. (4)) and in section E (Eq. (5)) were developed in the same way:

$$\frac{\overline{C}_{w(0-R/2)} - C_{we}}{C_{w0} - C_{we}} = 8\sum_{n=1}^{\infty} \frac{e^{-D_{we}\lambda n^2 t}}{(\lambda n R)^2 J_1(\lambda n R)} J_1(\lambda n \frac{R}{2}) \tag{4}$$

$$\frac{\overline{C}_{w(R-R/2)} - C_{we}}{C_{w0} - C_{we}} = \frac{16}{3}\sum_{n=1}^{\infty} \frac{e^{-D_{we}\lambda n^2 t}}{(\lambda n R)^2 J_1(\lambda n R)} \left[J_1(\lambda n R) - J_1(\lambda n \frac{R}{2}) \right] \tag{5}$$

The equilibrium moisture content was calculated from Peleg´s model (Peleg, 1988) and the effective diffusivities were estimated in the same way as for experiment I. The goodness of the fit was evaluated by means of %var.

2.5. Temperature effect on effective diffusivity

The Arrhenius equation was applied in order to determine the influence of temperature on water and nitrate diffusion coefficients. The accuracy of the fit was assessed through the p-value and R^2.

3. Results and discussion

3.1 Water content

The water content of fresh meat was 73% on wet basis. The evolution of the experimental average moisture content of the whole cylinder samples during the curing process for diffusion parallel to the meat fiber carried out at different temperatures is shown in Fig. 2a. As expected, the longer the curing time, the lower the moisture content. At the three experimental temperatures, it can also be observed that the initial decrease in water content occurs more quickly during the first 5 days of curing, followed by a slower decrease. The drop in the moisture content was faster for the samples salted at 12 and 7°C than for those salted at 2ºC; this is due, among other things, to the higher water activity of the $NaNO_3$ saturated brine at 2 ºC, which reduced the osmotic dehydration of the samples. Despite the fact that an increase in temperature in the initial steps of curing could accelerate the water loss, it is constrained due to its negative effects on microbiological stability. The water content in the samples cured at 2, 7, and 12°C was 1.20, 0.81 and 0.75 kg water/kg dry matter, at 16 (2ºC) and 11 (7ºC and 12ºC) days, respectively. Similar trends were obtained by Gómez et al. (2015) for pork meat cured at different temperatures with sodium nitrite ($NaNO_2$).

The effect of temperature on the average water content for diffusion perpendicular to meat fibers is shown in Fig. 3a. A similar temperature effect on curing kinetics was observed by Boudhrioua et al. (2009).

Fig. 4 shows the evolution of the experimental average moisture content of the two cylinder sections during the curing process for experiment II (diffusion perpendicular to meat fibers) carried out at different temperatures. The two cylinder sections showed a sharp decrease during the first 12 hours, thereafter remaining nearly constant. As expected, during this initial period, the external section of each cylinder in contact with the brine presented a higher dehydration than the internal one. At 8 and 12 ºC, it can also be observed that the initial decline in the moisture content was faster than at 0 and 4 ºC. The equilibrium water content was reached at between 1.5 and 2 days for the internal section and between 1 day and 1.5 days for the external section. The equilibrium moisture content was the same at every temperature and for both sections, with a value of 0.75 kg water/kg dry matter. When compared with the diffusion experiments parallel to the meat fiber, a similar equilibrium moisture content was obtained at 7 and 12ºC. The observed dehydration phenomenon typically occurs during the dry-curing of loin and ham, since salt penetration takes place simultaneously with water exit producing weight loss (García-Gil et al., 2014).

3.2 Nitrate content

The results for nitrate gain parallel to the meat fiber are shown in Fig. 5. As can be observed, the position affects the nitrate concentration. It is clear from Fig. 5 that the nitrate content of the meat cylinder slices increased in line with the curing time at all processing temperatures. It must be noted that, as expected, nitrate content values in slice A were higher at every studied temperature because this slice was in contact with the brine, while slice D, which is farther from the brine, had the lowest nitrate concentration. After 21 days of curing, the nitrate contents for slice A were 0.93, 1.62 and 2.19 g/L at 2ºC, 7ºC and 12ºC, respectively. In comparison, the nitrate contents in slice D after 21 days of curing were 0.009, 0.051 and 0.180 g/L at 2ºC, 7ºC and 12ºC, respectively. These results agree with the ones obtained by Gómez et al. (2015) for nitrite diffusion.

Fig. 6 shows the evolution of the experimental average nitrate content of the two cylinder sections during the curing process for experiment II carried out at different temperatures. A faster increase in nitrate gain was observed at every experimental temperature during the first 12 hours of curing, which was more marked in the external section. Wang et al. (2000) observed that NaCl diffusion behaved similarly, which was attributed to the large concentration gradient between brine and meat at the beginning of the salting process.

At the end of the studied period, the nitrate concentration in the internal and external section was similar, with values around 66.8 g/L at 0ºC, 88.6 g/L at 4ºC, 113 g/L at 8ºC and 124 g/L at 12ºC. This indicates a homogeneous distribution of the curing salt.

In order to study the effect of temperature on the average nitrate content of samples, the experimental average nitrate content of each cylinder was calculated and plotted versus time, both for the diffusion parallel to meat fibers (Fig. 2b) and for the perpendicular (Fig. 3b). As can be observed, the higher the temperature, the greater the average nitrate gain of the samples. These results are in line with those of Gómez et al. (2015) working on pork meat curing, Corzo et al. (2006) using sardine sheets and Telis et al. (2003) studying caiman muscle.

Figs. 7 and 8 show the amount of nitrate converted to nitrite for every experimental condition in this study. As can be observed, the transformation of nitrate into nitrite varies in line with curing time, temperature and position. The quantity of converted nitrate increased the longer the salting time went on, with the sections closest to the brine exhibiting the greatest conversion. Furthermore, a rise in temperature resulted in an increase in the conversion of nitrate to nitrite. However, this conversion was small in comparison to the nitrate uptake in both directions.

Tables 1 and 2 show the mean percentage of nitrate converted to nitrite parallel and perpendicular to the direction of the meat fiber, respectively. The reduction of nitrate to nitrite was lower perpendicular to fibers (with a maximum close to 0.5

%, as can be observed in Table 2) than parallel (maximum conversion 2.86% as observed in Table 1). This can be explained by the lower superficial water activity in the cylinders of experiment II. In that experiment, cylinders are totally immersed in the brine, which produces an environment that hinders the growth of microorganims with nitratereductase activity. Nevertheless, the percentage of reduction was low in both cases. For that reason, the conversion of nitrate to nitrite was not considered when modelling. This is a slow process that requires both long storage periods and the presence of microorganisms with nitrate reductase activity.

3.3. Mathematical modelling

Table 3 shows the average values of the effective diffusivity of nitrate and water. The diffusivities obtained in this study are of the same order as those found in the literature for other salts. Boudhrioua et al. (2009) studied the diffusion of sodium chloride and water in sardine fillets. These authors obtained values for the diffusion coefficient of water of between $2.4 \cdot 10^{-10}$ and $1.9 \cdot 10^{-8}$ m^2/s at temperatures of 5ºC to 20ºC. Graiver et al. (2006) reported values of between $0.6 \cdot 10^{-10}$ and $5 \cdot 10^{-10}$ m^2/s for the effective diffusion coefficient of NaCl in pork tissue salted in saturated brine (30 to 200 g/L) at 4ºC. When analysing the drying of *Semimembranosus* muscle in pork, Ruiz-Cabrera et al. (2004) obtained diffusivity values ranging from $3.45 \cdot 10^{-10}$ to $2.45 \cdot 10^{-9}$ m^2/s at between 12 and 20ºC. Sabadini et al. (1998) obtained a value of $2.5 \cdot 10^{-10}$ m^2/s for the diffusion coefficient of NaCl in beef in a saturated solution at 10ºC. Fox (1980) found a diffusion coefficient of $2.2 \cdot 10^{-10}$ m^2/s at 12ºC in the *Longissimus dorsi* muscle of pork immersed in a saturated salt solution (180 g/L).

Gómez et al. (2015) performed a similar experiment to study the nitrite and water diffusion in pork meat curing with sodium nitrite parallel to the meat fiber. The diffusion coefficients of nitrite ion obtained at temperatures of between 2ºC and 12ºC ranged from $0.04 \cdot 10^{-10}$ to $0.11 \cdot 10^{-10}$ m^2/s, which are higher than those obtained in the present study for nitrate. This can be explained by the fact that the nitrite ion presents a lower molecular weight than the nitrate, which facilitates the diffusion of nitrite into the meat (Marañón and Marañón 2005a, 2005b). On the other hand, the water diffusion coefficients obtained by the same authors ranged from $59.40 \cdot 10^{-10}$ to $97.73 \cdot 10^{-10}$ m^2/s, lower than the ones in this study.

The accuracy of the proposed models for the water and nitrate diffusion kinetics in both directions can be observed through the comparison of the experimental and simulated curing curves (Figures 2 and 3). A good fit was obtained between the experimental and calculated data for the water and nitrate content (%var > 90%). In every case, a good correlation coefficient was obtained for all of the temperatures ($r^2 > 0.98$). Thus, Fick's law properly describes the diffusion process in both directions.

3.4. Influence of temperature

From the diffusivity values shown in Table 3, it can be observed that temperature has a significant influence on the effective diffusivity ($p < 0.05$). Other authors reported a similar effect of temperature on the effective diffusivity of salts, such as sodium nitrite (Gómez et al., 2015), a mixture of curing salts (Pinotti et al., 2002), or sodium chloride (Chiralt et al., 2001; Telis et al., 2003). Moreover, this effect has also been observed in the effective diffusivity of water in meat (Gómez et al. 2015, Corzo and Bracho, 2008; Gou et al., 2003; Clemente et al., 2007).

Although a rise in temperature from 2 to 12 ºC increases the diffusion coefficient of nitrate and water, it also increases the microbial risk during curing and subsequent resting, especially in bone-in meat products, such as hams. Afterwards, this risk decreases during the subsequent industrial drying step due to the water activity reduction.

The activation energy values for the diffusion of nitrate (E_{Na}) and water (E_{wa}) were calculated by means of the Arrhenius equation. The E_{wa} and E_{Na} for diffusion parallel to the meat fiber were 15.06 kJ/mol (R^2 = 0.94, p-value = 0.0031) and 96.44 kJ/mol (R^2 = 0.99, p-value = 0.0001), respectively; while perpendicularly, they were 31.86 kJ/mol (R^2 = 0.91, p-value = 0.002) and 24.71 kJ/mol (R^2 = 0.96, p-value = 0.0001). This represents an indication that water and nitrate diffusion behave differently depending on the direction of the meat fiber.

These results coincide with those found in the literature. Other E_{wa} data reported are: 25.94 - 61.65 kJ/mol for *Gluteus Medius* muscle salted with NaCl (Gou et al., 2003), 27.8 kJ/mol for *Biceps femoris* and *Semimembranosus* muscles salted with NaCl and dried (Clemente et al., 2007) or 22 kJ/mol for pork meat salted with NaCl (Palmia et al., 1993). The reported E_{Na} values are 60.32 kJ/mol for nitrite in meat pork curing (Gómez et al., 2015) and sodium chloride in different fish and caiman, with values ranging from 29.00 to 168.13 kJ/mol (Corzo and Bracho, 2008; Telis et al., 2003; Uribe et al., 2011; Zhang et al., 2011).

In this study, the activation energy value for parallel nitrate diffusion (96.44 kJ/mol) is higher than that obtained by Gómez et al. (2015) for nitrite diffusion in the same direction (60.32 kJ/mol). As previously stated, the nitrite diffusion coefficient is higher than that of nitrate, which translates into a lower activation energy.

3.5. Influence of the direction of the meat fiber

The results presented in this study show that nitrate and water diffusion behave differently depending on the direction of the meat fiber, which points to the anisotropy of *Semimembranosus* muscle. In fact, Table 3 shows that the effective diffusivity for water is greater when parallel to the fiber than when perpendicular, as is also the case for water mobility. The opposite is observed for nitrate transport, since the nitrate mobility parallel to the fiber is lower. These results are comparable to those of Gou et al. (2002). When analyzing salted ham muscle, these authors observed that water diffusion coefficients perpendicular to the meat fiber were lower than the ones parallel to it. Thorvaldsson and Skjöldbrand (1996) found that water transport while cooking beef was about 20-25% slower when perpendicular to the fiber. These authors suggested that water can move straightforwardly parallel to the fiber. Nonetheless, perpendicular to the meat fiber, water has to move around both the fiber and the fiber bundles, which makes the path longer. This also happens in the curing process, affecting the nitrate movement. When curing parallel to the meat fiber, the faster movement of water produces greater tissue dehydration along the fiber, limiting the movement of nitrates. Due to their high degree of solubility, nitrates are mobilized into the meat fiber in the aqueous phase (Hönikel, 2008) through the inter-myofibrillar water and the inter-fascicular water, since the largest proportion of meat water, about 85%, is located in these zones (Pearce at. al, 2011). Thus, nitrate transport is slower when parallel to the meat fiber. These results are linked to those of Costa-Corredor et al. (2010), who found that the diffusivities of salts in pork meat are heavily dependent on the water content. These phenomena could be explained in terms of water-structure maker or water-structure breaker effect of different ions on the solvent, although the microscopic origins of these features have remained elusive (Poulanne and Halonen, 2010). Nevertheless, the results are of particular interest in bone-in meat products, such as dry-cured ham, in which some muscles present diffusion parallel to meat fibers (e.g. *Adductor*), while in others, the diffusion is perpendicular to them (e.g. *Gracilis*).

The E_{wa} perpendicular to the meat fiber was higher than when parallel to it, indicating that water needs more energy to diffuse perpendicularly to the meat fiber than parallel to it, which again confirms the muscular anisotropy. On the contrary, the E_{Na} parallel to the meat fiber was higher than when perpendicular to it, which agrees closely with the above-mentioned discussion.

4. Conclusions

The kinetics profiles and modelling results confirm that the transport and distribution of nitrate and water into meat samples can be analyzed by considering muscle orientation (unsteady bidirectional diffusion). A close agreement was found between the experimental kinetics (water loss and nitrate gain) and the diffusion models. These results revealed that curing was slower when nitrate was transported parallel to the direction of the meat fiber than when transported perpendicularly to it, as confirmed by the obtained diffusion coefficients. The E_{wa} for diffusion carried out perpendicularly to the meat fiber was higher than when parallel to it. On the contrary, the E_{Na} obtained for diffusion parallel to the meat fiber was higher than when performed perpendicularly to it.

These findings can help meat industry to a better management of the curing process with the aim of developing healthier meat products.

Nomenclature

C	Concentration of nitrate or water	kg/m^3
C_e	Equilibrium concentration of nitrate or water	kg/m^3
$\bar{C}_s$	Average nitrate concentration	kg/m^3
C_{se}	Average equilibrium nitrate concentration	kg/m^3
C_{s0}	Average initial nitrate concentration	kg/m^3
$\bar{C}_w$	Average moisture content	kg water/kg dry matter
C_{we}	Average equilibrium moisture content	kg water/kg dry matter
C_{w0}	Average initial moisture content	kg water/kg dry matter
C_0	Initial concentration of nitrate or water	kg/m^3
D_e	Effective diffusivity of nitrate or water	m^2/s
D_{Ne}	Effective diffusivity of nitrate	m^2/s
D_{we}	Effective diffusivity of water	m^2/s
E_{Na}	Activation energy for nitrate	kJ/mol
E_{wa}	Activation energy for water	kJ/mol
R	Radius of the cylinder	m
t	Time	s
$\%var$	Percentage of explained variance	

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Table captions

Table 1. Mean percentage (%) of nitrate converted to nitrite. Diffusion parallel to the direction of the meat fiber.

Table 2. Mean percentage (%) of nitrate converted to nitrite. Diffusion perpendicular to the direction of the meat fiber.

Table 3. Values of the effective diffusivity and standard deviation (sd) of nitrate and water both parallel and perpendicular to the meat fiber at different temperatures. Different letters in the same column indicate significant differences ($p < 0.05$).

Tables

Table 1.

Temperature (ºC)	Salting time (days)	Section			
		A	B	C	D
2	1	1.33	0.14	0.02	0.01
	3	1.21	0.14	0.02	0.01
	7	0.84	0.24	0.09	0.01
	11	0.71	0.29	0.06	0.02
	16	0.60	0.31	0.05	0.02
	21	0.48	0.37	0.06	0.05
7	1	0.14	0.05	0.05	0.05
	3	3.34	1.78	0.18	0.12
	7	3.76	2.84	0.96	0.20
	11	3.60	2.64	1.13	0.22
	16	3.00	2.34	0.96	0.36
	21	2.56	2.36	0.82	0.29
12	1	1.26	0.75	0.19	0.06
	3	2.83	1.37	0.26	0.11
	7	4.61	3.74	0.41	0.12
	11	3.67	2.80	0.73	0.41
	16	3.42	2.96	0.91	0.41
	21	2.86	2.47	1.20	0.45

Table 2.

Temperature (ºC)	Salting time (days)	Section	
		Internal	External
0	1	0.11	0.16
	2	0.12	0.16
	3	0.12	0.18
	4	0.13	0.19
	5	0.20	0.29
4	1	0.11	0.18
	2	0.11	0.16
	3	0.14	0.18
	4	0.15	0.20
	5	0.19	0.26
8	1	0.12	0.16
	2	0.10	0.16
	3	0.13	0.18
	4	0.17	0.22
	5	0.21	0.28
12	1	0.14	0.21
	2	0.22	0.31
	3	0.25	0.34
	4	0.28	0.38
	5	0.34	0.47

Table 3.

Diffusion parallel to meat fiber						
Temperature (ºC)	$D_{Ne} * 10^{10}$ (m^2/s)	sd $*10^{10}$	% var	$D_{we} * 10^{10}$ (m^2/s)	sd$*10^{10}$	% var
2	0.007^{a}	0.092	95.52	98.7^{A}	0.01	90.11
7	0.017^{b}	0.064	95.14	109.6^{B}	0.72	93.61
12	0.034^{c}	0.304	95.12	124.6^{C}	0.33	91.05
Diffusion perpendicular to meat fiber						
Temperature (ºC)	$D_{Ne} * 10^{10}$ (m^2/s)	sd$*10^{10}$ $* 10^{10}$	% var	$D_{we} * 10^{10}$ (m^2/s)	sd$*10^{10}$	% var
0	0.89^{d}	0.079	90.1	52.2^{D}	0.781	97.42
4	1.08^{e}	0.021	93.2	68.2^{E}	0.042	97.99
8	1.23^{f}	0.007	94.5	84.6^{F}	0.106	97.23
12	1.41^{g}	0.007	94.4	92.9^{G}	0.007	97.56

Figure captions

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.

Fig. 2. Kinetics of water loss (a) and nitrate gain (b) and fit of model to experimental data. ■ 2ºC, ▲7ºC, ● 12ºC and — model. Diffusion parallel to the direction of the meat fiber.

Fig.3. Kinetics of water loss (a) and nitrate gain (b) and fit of model to experimental data. ◊ 0ºC, X4ºC, □8ºC, ○ 12ºC and — model. Diffusion perpendicular to the direction of the meat fiber.

Fig. 4. Kinetics of water loss in cured cylindrical samples. a) Internal cylinder, b) External cylinder. Diffusion perpendicular to the direction of the meat fiber. ◊ 0ºC, ж 4ºC, ○ 8ºC and □ 12ºC.

Fig. 5. Kinetics of nitrate gain at different temperatures: (a) 2ºC, (b) 7ºC and (c) 12ºC. ♦ slice A, ■ slice B, ▲slice C and ж slice D. Diffusion parallel to the direction of the meat fiber.

Fig. 6. Average experimental nitrate content of samples versus salting time : (a) Internal cylinder, (b) External cylinder. ♦ 0ºC, x 4ºC, ▲8ºC, ■ 12ºC. Diffusion perpendicular to the direction of the meat fib

Fig. 7. Nitrate converted to nitrite versus salting time: (a) 2ºC, (b) 7ºC and (c) 12ºC. ♦ slice A, ■ slice B, ▲slice C and ж slice D. Diffusion parallel to the direction of the meat fiber.

Fig.8. Nitrate converted to nitrite versus salting time: (a) Internal cylinder, (b) External cylinder. ♦ 2ºC, x 4ºC, ● 8ºC and ■ 12ºC. Diffusion perpendicular to the direction of the meat fiber.

Figures

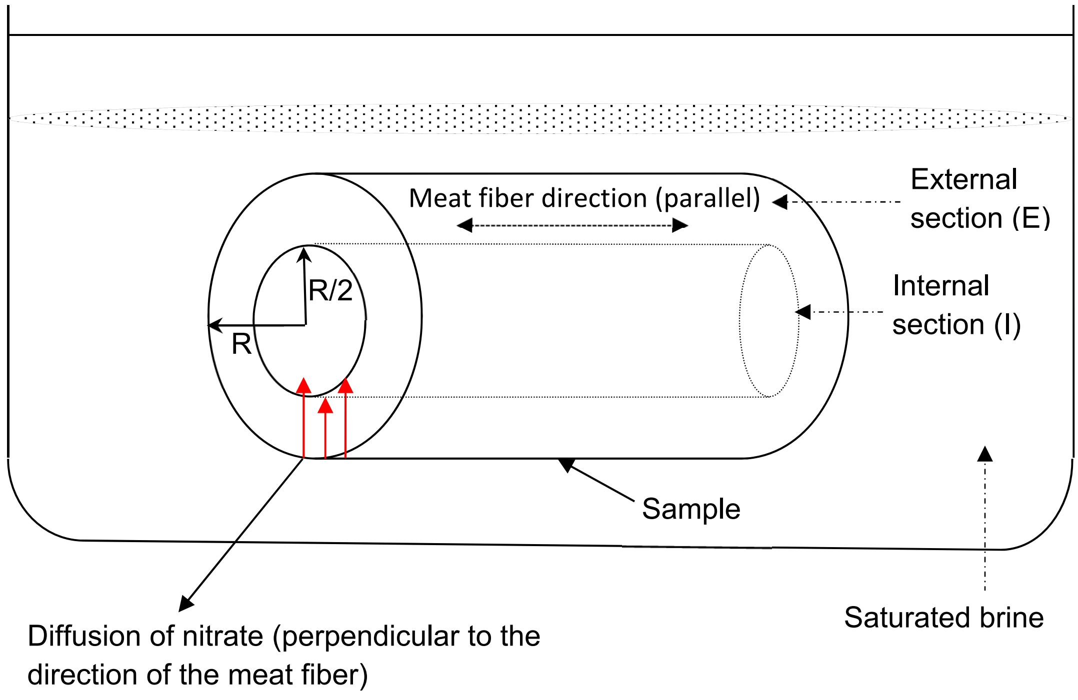


Fig. 1.

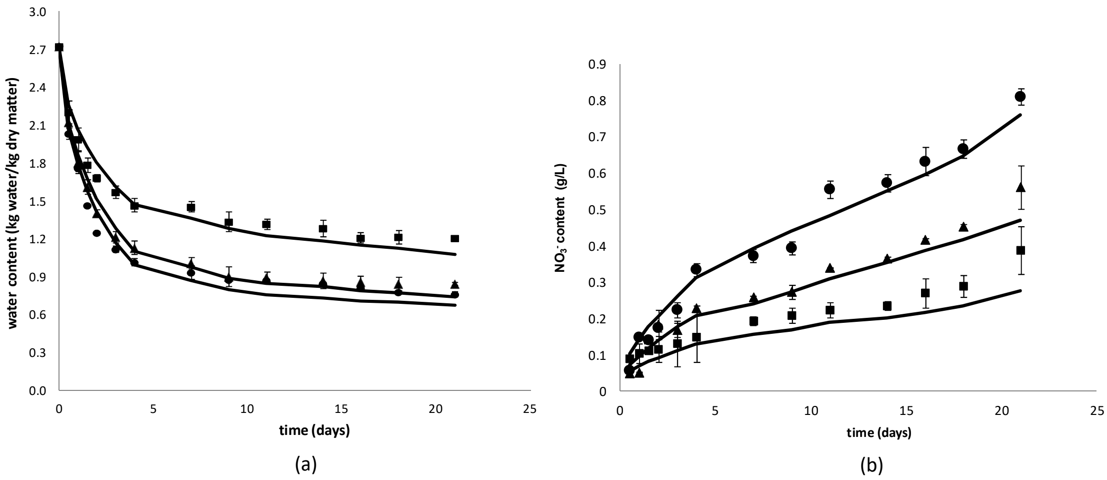
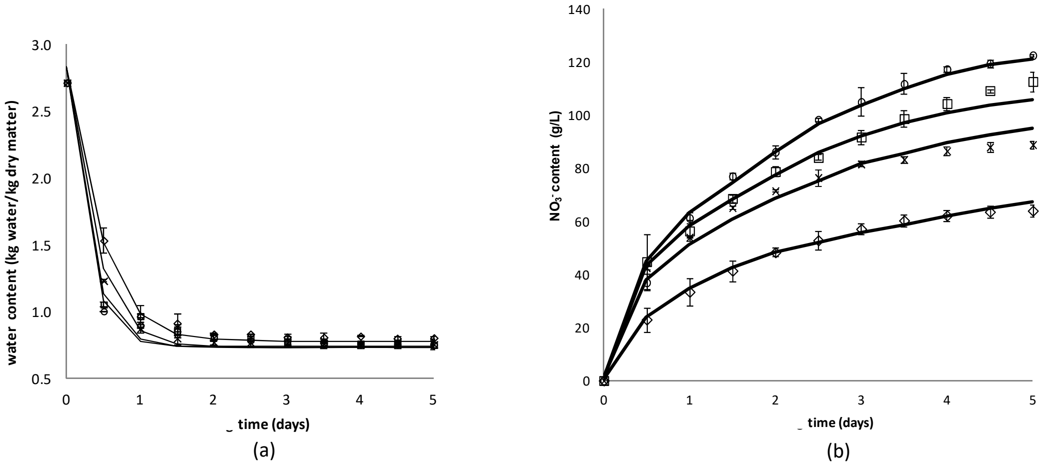
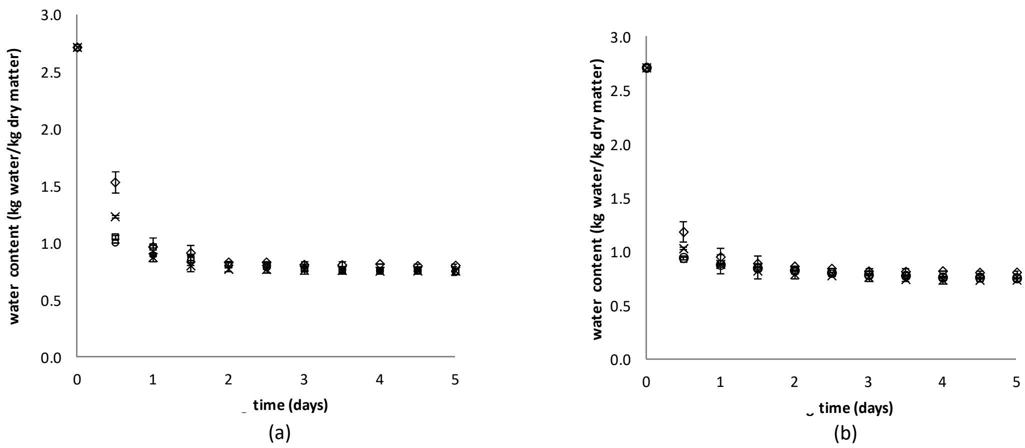

water content (kg water/kg dry matter)
time (days)
(a)
NO3- content (g/L)
time (days)
(b)

Fig. 2.



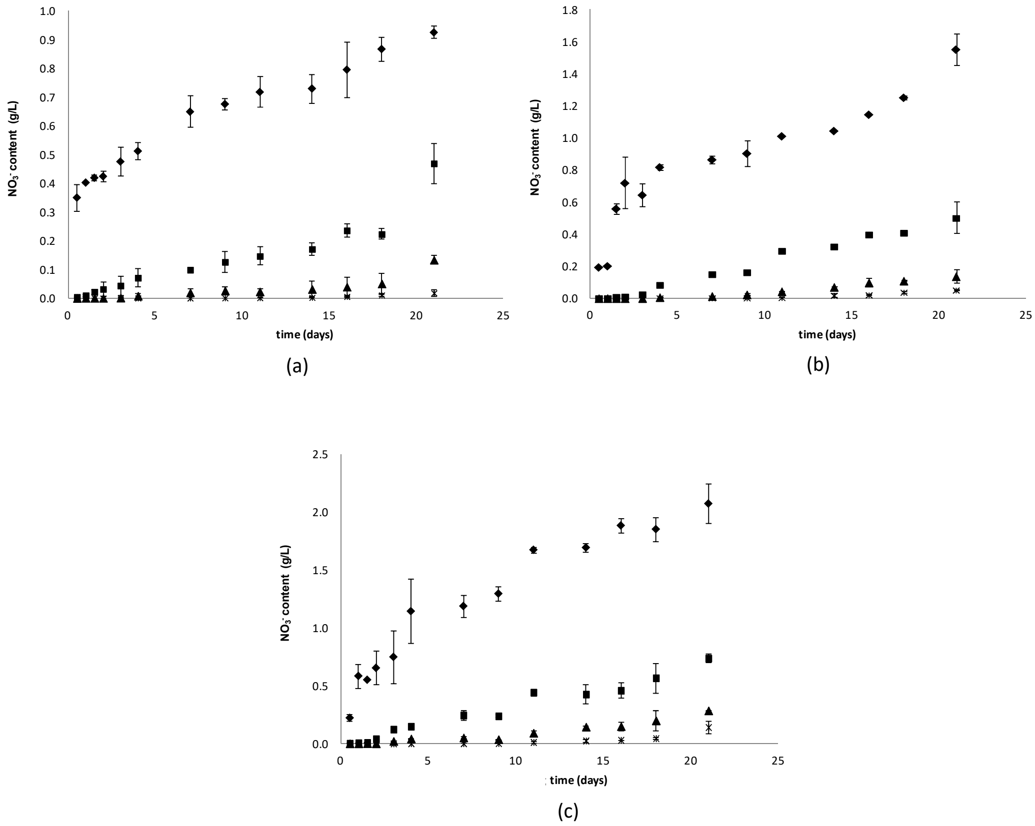
water content (kg water/kg dry matter)
time (days)
(a)
NO3- content (g/L)
time (days)
(b)

Fig.3.



water content (kg water/kg dry matter)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
time (days)
(a)
water content (kg water/kg dry matter)
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
1
2
3
4
5
time (days)
(b)

Fig. 4.



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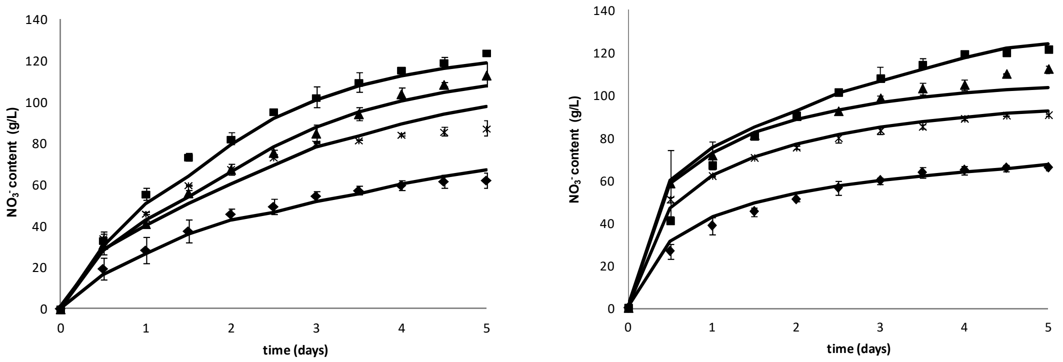
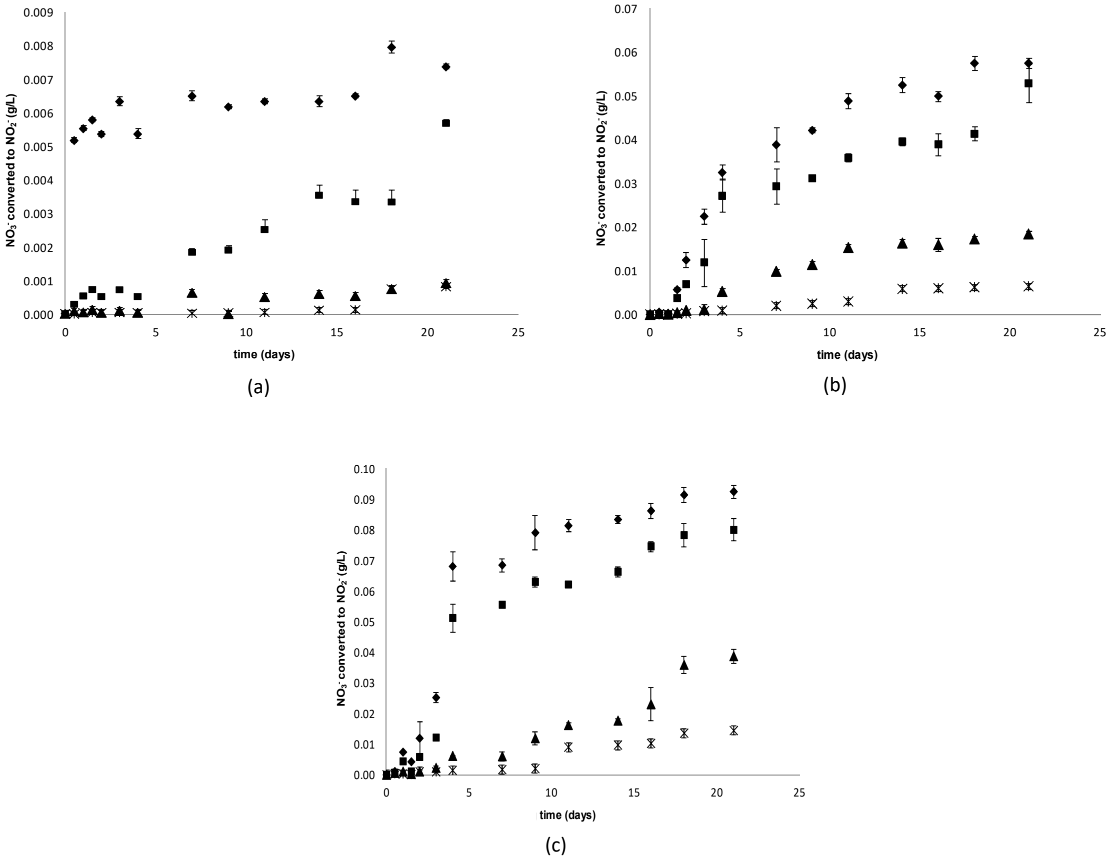


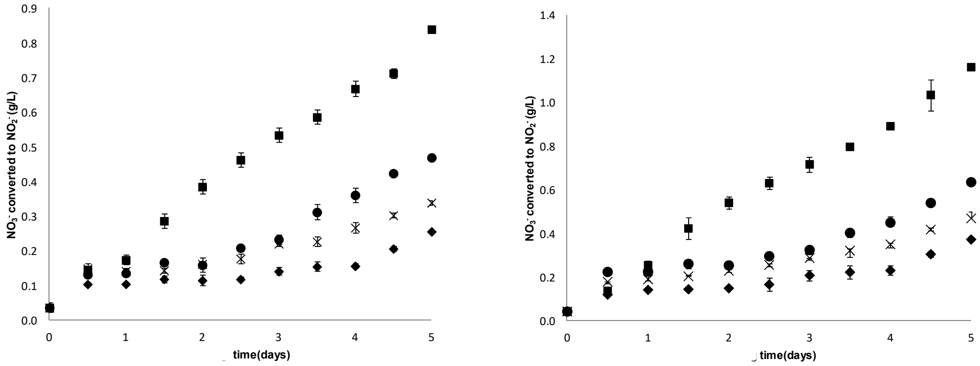
(a) (b)

Fig. 6.



NO_3^- converted to NO_2^- (g/L)
time (days)
0.000
0.001
0.002
0.003
0.004
0.005
0.006
0.007
0.008
0.009
0
5
10
15
20
25
(a)
NO_3^- converted to NO_2^- (g/L)
time (days)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0
5
10
15
20
25
(b)
NO_3^- converted to NO_2^- (g/L)
time (days)
0.00
0.01
0.02
0.03
0.04
0.05
0.06
0.07
0.08
0.09
0.10
0
5
10
15
20
25
(c)

Fig. 7.



(a) (b)

Fig.8.