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Assessment of bacterial growth on the surface of meat under common processing conditions by combining biological and physical models

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Abstract

A water transfer model and a bacterial model were combined to study the effects of process parameters (air temperature, velocity and relative humidity (RH)) on the drying of the food surface and their indirect consequences on bacterial growth. They were tested on experimental growths of *Pseudomonas* spp. inoculated on pork meat: small variations in a parameter can have a considerable effect on bacterial growth. Sensitivity calculations showed how the meat properties (diffusivity, sorption isotherm, thickness) affected the calculated results. The combined models were applied to study the impact of air velocity and RH on the increase in the bacterial population after 96 h of storage at 12 °C. Thus, no more than a two log unit increase is obtained (1) if the air velocity is equal to 0.2 m/s and RH below 82% or (2) if RH is as high as 90%, air velocity must be equal to 0.9 m/s.

Keywords: Surface water activity; Predictive microbiology; Meat; Water mass transfer; Modelling

1. Introduction

The safety and quality of foodstuffs depend on both raw materials and processing procedures. When operations like heating or dehydration are applied to stabilise or transform food products, the process conditions partly determine the microbial quality (Chirife & Ferro Fontan, 1982; McDonald & Sun, 1999). For example, in several air treatment processes such as cold storage, ripening of cheese or maturation of fermented sausages, the microbial growth on the product surface is known to closely depend on air properties: temperature (T), velocity (v) and relative humidity (RH).

It is nevertheless impossible to determine the rules governing the interactions between these process control variables and the final microbial quality from the analysis of experimental data because the true variables which affect microbial growth are the surface temperature and water activity (a_w). Their values cannot be easily derived from the air temperature, velocity and relative humidity because the product surface is not in equilibrium with the air. Moreover, the values of these process variables often vary with time. The surface temperature and water activity are the results of the balances of heat and water transfers which take place both inside the product and at the air/product interface. The physico-chemical properties of the product are important parameters as well.

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Since all of the many possible industrial situations cannot be reproduced in the laboratory and analysed, a mathematical modelling strategy was developed to investigate how process variables affect microbial growth.

This study has focused on water transfer and change in surface water activity ($a_{w,s}$) for two reasons: (1) the $a_{w,s}$ cannot be directly measured, and (2) studies in predictive microbiology have shown that bacterial growth rates greatly vary in the narrow range of a_w between 0.9 and 1 (Cheroutre-Vialette & Lebert, 2002; McClure, 1999; Thompson, Busta, & Schmidt, 1986).

Complementary studies had already been performed in our laboratory prior to this one:

- Microbial predictive models were developed to predict bacterial growth as a function of a_w (Lebert, Robles-Olvera, & Lebert, 2000).
- A new device and method (Baucour & Daudin, 2000) was established to measure sorption isotherms of solid foods in the high humidity range to which bacterial growth rate is very sensitive.
- Kondjoyan, Daudin, and Bimbenet (1993), Kondjoyan (2001) and Ghisalberti and Kondjoyan (2001) characterised the heat and mass transfer coefficients at the air/product interface under industrial process conditions.
- The water diffusivity (D) and its variation with water content were assessed from NMR 2D images of water distribution within samples of gelatine gel and pork lean meat undergoing drying (Ruiz-Cabrera, Daudin, Foucat, & Renou, 1998a, 1998b).

In order to test the proposed strategy in a case for which most of the needed data were available from our previous studies, this particular study focused on the growth of natural contamination of *Pseudomonas* spp. on pork meat. For the sake of simplicity, isothermal conditions were considered. Furthermore, these conditions approximately correspond to the above-mentioned processes.

A water transfer model was built in order to integrate the process variables (air v, T and RH) and the product characteristics (water sorption and diffusivity). The temperature fluctuations due to evaporation were minimal and neglected. The evolution of the water activity on the surface of meat was derived from this model and, as a result, used to estimate the *Pseudomonas* growth under dynamic conditions using a microbial predictive model. A lab-scale experiment duplicating industrial conditions and carried out under well-controlled conditions was designed to compare the measured bacterial growth to the predictions. Some calculations were also performed to analyse the magnitude of the effect of some parameters.

2. Materials and methods

2.1. Strains

The growth of *Pseudomonas* spp. took place in naturally contaminated pork muscle since *Pseudomonas* rapidly becomes the dominant background microflora in meat stored at low temperatures.

2.2. Pork meat

Three *semi-membranosus* pork muscles (pH = 5.7) were cut into samples of 3×3×2 cm. Four samples where kept for water sorption isotherm measurements and sixty were used in the bacterial growth experiments. These latter were mixed in a laminar airflow cabinet (NU-425-400, Nuaire, Vie 3000, Balan, France) in order to homogenise the natural contamination.

2.3. Sorption isotherm curves

The sorption isotherms were measured in the high humidity range ($0.85 < a_w < 1$) with the device and method established by Baucour and Daudin (2000). Ten thin slices—1 g each—removed from the above samples were placed in the 10 equilibrium cells (12 °C but at different relative humidity values) and submitted to a very high velocity air flow (more than 10 $m\,s^{-1}$) which allows an equilibration time of less than 48 h. The relative humidity in each cell was controlled by a reduction in pressure of the initially saturated air. This experiment was carried out four times to check repeatability.

2.4. The wind tunnel

In a wind tunnel described by Robles-Olvera, Bégot, Lebert, and Lebert (1999), bacterial growth can be measured on the surface of food products under controlled conditions of temperature, air velocity and RH. The wind tunnel is automated and equipped with sensors: platinum probes for air temperature and dew point sensors for RH.

2.5. Bacterial growth experiments

Two kinds of experiments were performed at 12 °C:

(1) a Petri dish experiment to verify if the model of Lebert et al. (2000) could be applied to predict bacterial growth on pork meat. Meat samples were incubated in Petri dishes in which relative humidity (RH) close to 100% was sustained throughout the experiment (Lebert, Bégot, & Lebert, 1998);
(2) a validation experiment with the wind tunnel (i.e. close to industrial conditions) for comparison with the calculations. Sixty meat samples were placed in

a tray which was placed in the drawer of the wind tunnel. The relative humidity was controlled so that the effect of $a_{w,s}$ changes on the growth of *Pseudomonas* could be evaluated.

During the validation experiment, the samples were placed side by side to form a 'flat plate', dried on one side by the air flow. To avoid any effect of sample position on bacterial growth, the samples were randomly chosen at each sampling time for microbial analysis. To maintain the 'flat plate' system, the removed samples were replaced by parallelepipeds made of stainless steel of the same size. At each sampling time, two meat samples were analysed for viable counts. Only the surface of the sample in contact with air was removed with a scalpel and numbered.

Each sample was plated in duplicate using a spiral plater (DS, Interscience, Saint Nom la Bretèche, France). Bacterial counts were performed (incubation temperature): on tryptone soy agar (TSA) (24 °C) for non-selective counts to control the level of background microflora and on *Pseudomonas* Agar Base (24 °C) (CFC, Oxoïd, Unipath Ltd., Basingstoke, England), supplemented with *Pseudomonas* CFC Supplement (Oxoïd), for *Pseudomonas* spp. The pH was determined at alternate sampling time points using a pH-meter probe (Ingold, Bioblock Scientific, Illkirch, France).

3. Mathematical models

3.1. Water transfer model

The water transfer inside meat was modelled using a unidirectional and isothermal Fickian diffusion model. The major problem encountered when simulating the behaviour of foodstuff during air drying is its shrinkage which is not uniform. The higher the local water removal, the higher the local shrinkage. Thus, this phenomenon should be very prominent close to the surface where the a_w has to be derived from the predicted water content. The standard Fick equation which relates the water content to time and space was rewritten in Lagrangian coordinates or solid coordinates (Rovedo, Suarez, & Viollaz, 1998). In this case, the spatial referential remains constant since the water content is a function of ξ the 'length of dry matter':

$$\frac{\partial X}{\partial t} = \frac{\partial}{\partial \xi}\left(\left[\frac{D(X)}{(1+\varepsilon.X)^2}\right]\cdot\frac{\partial X}{\partial \xi}\right) \tag{1}$$

where t is the time (s), ξ is the Lagrangian coordinate (m), X is the water content on a dry basis (kg water/kg dry matter) and D is the water diffusivity (m^2/s). The volumetric shrinking coefficient ε is equal to the ratio of the dry matter density to that of water. In other words, it is assumed that the local decrease in volume is exactly equal to the decrease due to water removal.

At the air/product interface, water evaporation was taken into account through a third kind of convective boundary condition:

$$\Phi_W = k(P_{sat}\cdot a_{w,s} - P_a) \tag{2}$$

where Φ_w is the flux of water (kg water/m^2 s), k is the mass transfer coefficient (m/s) which depends on the air velocity, P_{sat} (Pa) is the saturated water vapour pressure at the product temperature and P_a (Pa) is the partial air vapour pressure which is a function of air temperature and relative humidity.

The water balance over the first thin layer below the surface is paramount for calculating the variation in water content which is used to calculate the activity at the surface from the water sorption curve:

$$\left[\frac{D(X)}{(1+\varepsilon\cdot X)^2}\right].\left.\frac{\partial X}{\partial \xi}\right|_{Surface} - \Phi_W = \left.\frac{\partial X}{\partial t}\right|_{Surface} \tag{3}$$

None of the physical properties which appear in Eqs. (1)–(3) were adjusted. The density of the dry matter was assumed to be equal to 1381 kg/m^3 (Miles, Van Beek, & Veerkamp, 1983). The relationship between the water diffusivity (D) and the moisture content (X) (Eq. (4)) was fitted from the results of Ruiz-Cabrera et al. (1998a) who determined D for various water contents from the analysis of NMR moisture distribution images measured during the drying of gelatine and meat samples.

$$D = a\cdot e^{(c+b\cdot X\cdot e^{(d\cdot(X+f))})}\cdot(a\cdot X+b) \tag{4}$$

where X water content of the gel (kg water/kg dry matter).

The parameter values in Eq. (4) were: $a=1.11$; $b=8.37\times10^{-5}$; $c=-25.39$; $d=0.31$; $f=25.85$.

Water diffusivity increases with water content from 2×10^{-11} m^2/s at 1 kg water/kg dry matter to a maximum of 10^{-9} at the initial water content (3 kg water/kg dry matter). The mass transfer coefficient (k) was measured in independent experiments using a psychrometric method (Kondjoyan & Daudin, 1993) and the heat transfer coefficient was calculated by using the Lewis relation (Ozisik, 1985). At an air velocity of 2.3 m/s, the value of k was found equal to 0.0218 m/s.

The program was designed using Matlab 5.2 software. The resolution was based on an explicit finite difference scheme with centrally located differences. In order to obtain a good level of accuracy, a series of calculations were performed by refining the spatial discretisation until the results did not vary. It was finally set at 0.05 mm, i.e. 20 points per millimetre. The time step was automatically adapted to obtain convergence and minimise the calculation time which was equal to a few hours.

The transfer model had been validated on drying experiments using gelatine samples (30×10×15 mm) with an initial water content and pH similar to that of meat (3 kg water/kg dry basis and 6.0) and placed in an air flow with $v = 0.56$ m/s and RH = 40% (Baucour, Ruiz-Cabrera, & Daudin, 1999). The consistency between the measured and calculated moisture profiles demonstrated the soundness of the model even though no physical parameters were adjusted.

Three kinds of simulation calculations were performed. The model was used (i) to assess the $a_{w,s}$ during the validation drying experiment on pork lean meat, (ii) to investigate the influence of some variables on the course of the $a_{w,s}$ to support the discussion, and (iii) to analyse the combined effect of air velocity and humidity on meat storage.

3.2. Prediction of growth under dynamic conditions

The growth model of *Pseudomonas* spp. developed by Lebert et al. (2000) is composed of two parts:

- the primary model which expresses the evolution of the bacterial population with time, is the modified Gompertz equation (Eq. (5)) (Zwietering, Jongenburger, Rombouts, & Van't Riet, 1990):

$$y(t) = \mathrm{Log}_{10}\left(\frac{N}{N_0}\right) = A \cdot \exp\left(-\exp\left(\frac{\mu \cdot e}{A} \cdot (L - t) + 1\right)\right) \quad (5)$$

  where A is the logarithmic increase of the bacterial population, L is the lag time, μ is the maximal growth rate (on Log_{10} basis), t is the time and N_0 is the initial population. Generation time (GT) was derived from the maximal growth rate: $\mathrm{GT} = \mathrm{Log}_{10}(2)/\mu$.
- the secondary model which expresses the growth parameters (lag time and maximal growth rate) under constant environmental conditions as a function of temperature, pH and a_w, is the polynomial model of Lebert et al. (2000) in which the correlations were made using the following terms: Constant, T, pH, Salt, T^2, pH^2, Salt^2, $T\times\mathrm{pH}$, $T\times\mathrm{Salt}$, $\mathrm{pH}\times\mathrm{Salt}$, where T: temperature (°C), Salt: NaCl%. The terms of the three *Pseudomonas* models are given in Table 1. The data of Chirife and Resnik (1984) were used to transfer NaCl% to a_w.

To model the growth of *Pseudomonas* spp. submitted to fluctuating a_w, similar hypothesis as for temperature (Zwietering, De Wit, Cuppers, & Van't Riet, 1994) were used:

- Hypothesis 1: if bacteria are subjected to an a_w change during the lag phase, they will not have completed their lag period and will still show a lag phase at the new a_w. It is supposed that the effect of the fluctuating condition results in a new lag that is equal to the relative part of the lag phase that still has to be completed.
- Hypothesis 2: if the a_w change occurs within the exponential phase, it is assumed that the bacteria show no surplus lag phase due to a change in a_w during the exponential phase, growth continues immediately at the growth rate associated with the new a_w.

To take the time variation of $a_{w,s}$ into account, the evolution of the $a_{w,s}$ was discretised into time intervals (Δt) by considering a fixed variation of water activity ($\Delta a_{w,s}$). An $\Delta a_{w,s} = 0.002$ was found to be the best interval for representing $a_{w,s}$ curves (Baucour, 2000).

The polynomial *Pseudomonas* model (Lebert et al., 2000) was used to calculate the growth parameters (i.e. L, GT) at 12 °C, pH 5.7 and $a_{w,s}$ for each time step (Δt). At each step, hypothesis 1 and 2 were checked to determine the state of the bacterial population: Lag or growth. In this last case, the bacterial population $N(t)$ at the end of the time interval was calculated from the population at the beginning, $N_0(t)$ using the primary model. The maximal population was set at 9.5 log (Colony Forming Unit).

Table 1
Parameters of the generation time (GT (h)) and Lag (L (h)) models developed for three *Pseudomonas* strains (*Pfr*: *P. fragi*; *Pfl*: *P. fluorecens*; Salt: % NaCl)

Parameters	*Pfr*162		*Pfr*K1		*Pfl*58	
	GT	L	GT	L	GT	L
Constant	4.6878	4.3606	4.3663	1.5554	8.0793	0.8679
T	−0.1766	−0.1093	−0.2161	−0.0353	−0.1875	−0.2723
pH	−1.1050	−0.9334	−0.8747	−0.3312	−2.0859	0.4593
Salt	0.2706	0.2069	0.1649	0.4389	0.1437	0.3567
T^2	0.0026	0.0015	0.0023	0.0002	0.0049	−0.0029
pH^2	0.0855	0.0737	0.0603	0.0379	0.1641	−0.0677
Salt^2	0.0164	0.0061	0.0207	−0.0089	0.0154	−0.0011
$T\times\mathrm{pH}$	0.0129	0.0001	0.0195	−0.0055	0.0074	0.0445
$T\times\mathrm{Salt}$	−0.0013	0.0012	−0.0035	−0.0013	−0.0010	−0.0103
$\mathrm{pH}\times\mathrm{Salt}$	−0.0440	−0.0155	−0.0301	−0.0311	−0.0235	−0.0107

3.3. Effects of process variables on a_w and bacterial growth

Simulations combining the water transfer model and the predictive microbial model were performed in order to study the influence of some process parameters on surface $a_{w,s}$ and the consequences on bacterial growth. The conditions taken to perform the calculations were: pork meat (pH 5.7, $Xo = 3$ kg water/kg dry matter, thickness = 2 cm), initial contamination of the meat by *Pseudomonas* spp. = 10 CFU/cm^2, the sorption isotherm of pork meat (Fig. 1), D variable with water content (measured by Ruiz-Cabrera et al. (1998a)), air temperature = 12 °C, air velocity = 2.3 m/s, the RH time variation of the validation experiment (Fig. 2). To perform a sensitivity analysis, these conditions were modified as presented in Table 2.

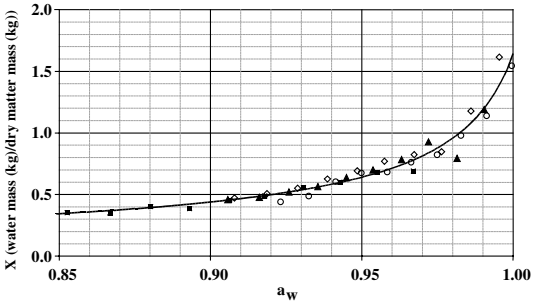

Fig. 1. Experimental sorption isotherms (four repetitions: ○, ◆, ■, ▲) at 12 °C made on pork *semi-membranosus* muscle and fitted curve with the Ferro Fontan model (—).

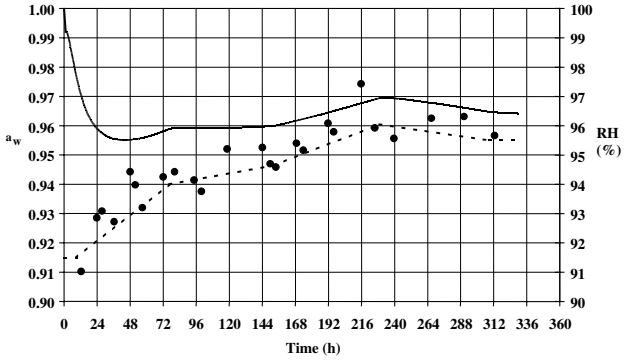

Fig. 2. Relative humidity (RH) in the wind tunnel during the validation experiment on pork meat at 12 °C, $v = 2.3$ m/s. Experimental RH measured with a Dew point sensor (●) and the corresponding fitted curve (---) used to calculate the a_w at the surface of pork meat (—).

Table 2
Tested variables and conditions applied for the simulations

Variables	Modified conditions
Water diffusivity	Constant (2×10^{-10} m^2/s) or variable with X
Sorption curve	±20% variation of X at a given a_w
Product thickness	From 0.5 to 5 cm
Air velocity	From 0.2 to 5 m/s
Air RH and velocity	RH from 70% to 98% and v from 0.1 to 1 m/s

Default conditions if not modified: Pork meat: pH 5.7, $X_0 = 3$ kg water/kg dry matter, thickness = 2 cm; Sorption isotherm of pork meat; $D = f(X)$; Air: 12 °C, $v = 2.3$ m/s, RH time variation of the validation experiment; Initial contamination of *Pseudomonas* spp.: 10 CFU/cm^2.

4. Results and discussion

4.1. Preliminary results

4.1.1. Sorption isotherms of pork meat

The water sorption isotherm measurements are presented in Fig. 1. a_w ranged from 0.9 to 1 for three of the experiments, in order to focus on high humidity conditions. Repeatability was very good. The range was extended to 0.85 in another experiment and all the experimental points were fitted with the Ferro Fontan relationship (Chirife, Boquet, Fontan, & Iglesias, 1983; Ferro Fontan, Chirife, Sancho, & Iglesias, 1982) which was shown to be a good fit for this asymptotic part of the isothermal sorption curves (Baucour & Daudin, 2000):

$$\ln\left(\frac{\gamma}{a_W}\right) = \alpha \cdot X^{-R} \tag{6}$$

where the adjusted parameters had the following values: $\alpha = 3.35\times10^{-2}$, $\gamma = 1.02$ and $R = 1.55$. Between $X = 3$ kg water/kg dry matter and $X = 1.6$ kg water/kg dry matter, the water activity of the meat was considered equal to 1.00.

4.1.2. Validation of the Pseudomonas spp. model on pork meat: Petri dish experiment

The *Pseudomonas* model has been already validated on beef meat (Lebert et al., 2000). It was tested here on naturally contaminated pork meat at an a_w close to 1 (Table 3). *Pseudomonas* spp. were considered to be the dominant microflora since the TSA and CFC counts were equal from the beginning of the exponential phase. Table 3 shows that the generation time of the experiment

Table 3
Experimental and predicted generation and lag times of growth experiments performed at 12 °C, $a_w \approx 1$, pH 5.7 and inoculum 2.1×10^5 CFU/cm^2

	A	GT (h)	L (h)
Experiment	4.5 (4.3–4.5)	2.5 (2.1–2.8)	10.8 (8.0–13.6)
Model *Pfr*162		2.0 (1.5–2.6)	2.2 (1.5–2.6)
Model *Pfr*K1		2.6 (1.8–3.6)	1.4 (0.0–4.5)
Model *Pfl*58		3.0 (2.0–4.7)	4.4 (1.9–10.3)

A, log increase in population; in brackets, confidence interval at 95%, Models (Lebert et al., 2000): *P. fragi* 162: *Pfr*162 (fast growth), *P. fragi* K1: *Pfr*K1 (slow growth), *P. fluorescens* 58: *Pfl*58 (slow growth).

was within the prediction range of the three models and was close to the *P. fragi* K1 model. Predicted lag times (L) are shorter than the experimental one but such results have already been observed on beef meat (Lebert et al., 2000). L is also known to be a parameter more difficult to predict and shows higher ratios of standard deviation/average (Lebert et al., 1998) and higher average errors between experimental and predicted values (Delignette-Muller, Rosso, & Flandrois, 1995) compared to the maximum growth rate ratios or average errors.

4.2. Validation experiments

4.2.1. Control of water activity and temperature on the surface of pork meat

In the study of Robles-Olvera et al. (1999), air velocity was set at 7 m/s and it was assumed that $a_{w,s}$ and RH rapidly reached equality at the surface of meat so that $a_{w,s}$ could be considered to be equal to RH/100. This assumption was confirmed later on in this study. During the validation experiment, the air velocity was reduced to promote drying conditions close to industrial conditions and for which there is no equality between $a_{w,s}$ and RH. It was set at 2.3 m/s. The time to reach the end of the growth, that is the maximal population, at 12 °C with an a_w of 0.96, was estimated at two weeks.

A series of calculations was performed with the water transfer model to find a good RH time-course to depress $a_{w,s}$. The RH was therefore set at 90% at the beginning of the experiment to dry out the surface before the bacterial growth reached the exponential phase. It was then slowly increased until it reached 95% during the first week to prevent the $a_{w,s}$ from dropping below the $a_{w,min}$ and was maintained at this value during the second week (Fig. 2). The $a_{w,min}$ was defined as the a_w below which the growth of *Pseudomonas* is inhibited. The tray on which the meat samples were placed was made of aluminium which is a good heat conducting material. The cooling effect of the surface water evaporation was negligible and the product temperature was maintained at air temperature.

Fig. 2 presents the physical conditions finally obtained during the validation experiment. The measured values of RH were averaged by successive linear regressions, as indicated by the dotted line, for input into the water transfer model. The predicted meat a_w actually decreased from 1 to 0.955 in 48 h, then slowly increased over 14 days until it reached 0.97.

4.2.2. Experimental growth results

The results are reported in Fig. 3 together with those obtained in the Petri dish experiment. In the validation experiment, it is clear that *Pseudomonas* growth was largely affected. Even if the initial bacterial contamination was different in each experiment (Fig. 3), it is clear that the growth in the validation experiment was three times

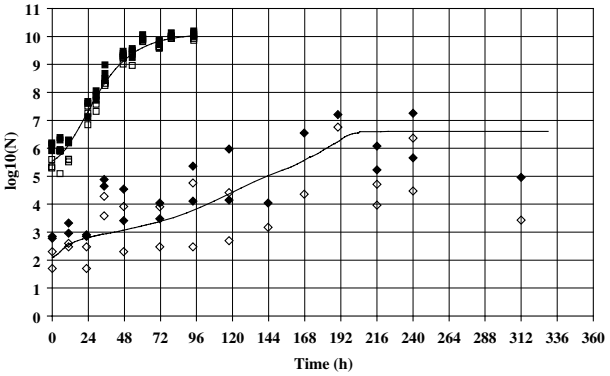

Fig. 3. Growth of *Pseudomonas* spp. at 12 °C on the surface of pork meat at pH 5.7 (a) Petri dish experiment at $a_w \approx 1$ (experimental counts: (■) TSA, (□) CFC) and (b) validation experiment at variable a_w presented in Fig. 2 (experimental counts: (◆) TSA, (◇) CFC). Comparison with *P. fragi* K1 model (—).

longer. This suggests that the $a_{w,s}$ was actually depressed, as expected, and maintained above the $a_{w,min}$. Nevertheless, the growth showed a large dispersion of the experimental points compared to the growth in the Petri dish experiment, regardless of the medium used, whether it be TSA or CFC agar. This cannot be explained by a variation in pH as the three muscles were similar in pH value (pH 5.7 ± 0.1). At some of the sampling times, flora other than *Pseudomonas* spp. (mainly cocci when observed with an optical microscope) had grown and become dominant, particularly at the end of the experiment. At each sampling time, two samples were removed and their positions were identified. No correlation was observed between the distribution of the samples and the dispersion. Moreover, for two samples removed at the same time, great differences were sometimes observed. For instance, at 144, 168 and 192 h, one sample had a bacterial CFC count lower than 100 CFU/cm^2, which is the detection limit of the counting method, while the other showed significant growth with a CFC count that was close to the TSA count.

All of the above observations suggest that the time variation of the $a_{w,s}$ was slightly different from one sample to another, sometimes decreasing below the $a_{w,min}$. This was probably due to the fact that these samples did not really make a perfect ideal flat slab. The difference in height between the pre-cut meat parallelepipeds which decreased during drying as well, and that of the stainless steel parallelepipeds used to replace the samples removed for analysis, promoted vortices that induced a variation in the mass transfer coefficient over the "slab" surface and therefore differences in the superficial drying out of the samples.

4.2.3. Predicted growth results

In the validation experiments, both $a_{w,s}$ and *Pseudomonas* growth were affected by the incubation conditions. The combination of the two models gave a

predicted growth curve in agreement with the growth observations (Fig. 3).

Zwietering et al. (1994) showed that the hypothesis used to model the lag phase with the modified Gompertz equation were accepted in more than 70% of the experiments with shift in temperature. They indicated that this assumption was more convenient for simulation purposes, especially if many temperature changes occurred during the incubation or if dynamically changing temperature occurred. Our study showed that this assumption can also be applied to dynamically changes in a_w.

4.3. Sensitivity studies

These results show that small variations in a parameter can have a considerable effect on bacterial growth. Thus, sensitivity calculations were performed according to the conditions presented in Table 2 and in particular by using the same air temperature and RH time-variation as in the validation experiment. The first aim was to investigate how the accuracy of the meat physical properties in relation to water transfer affected the calculated results. The second aim was to see if the samples used in the validation experiment were representative of large pieces of meat.

4.3.1. Effect of the accuracy of the product property values

4.3.1.1. Water diffusivity. In drying, it is often assumed that the water diffusivity is constant within the product because it is difficult and time consuming to assess the variation of the water diffusivity with the water content. Two conditions were tested. In the first one, the water diffusivity relationship was measured by Ruiz-Cabrera et al. (1998b). In the second one, the water diffusivity was considered equal to 2×10^{-10} m^2/s which was the average value measured between 0.5 and 3 kg water/kg dry matter. In the second case, the prediction (Fig. 4b) is at total odds with the results of the validation experiment (Fig. 3). Fig. 4a and b show that big differences are observed between the two conditions. The variations of the water diffusivity in the product must be taken into account in the combined model to correctly predict the bacterial growth.

4.3.1.2. Sorption curve. Fig. 5 shows the results for a variation of +20% or −20% of the water content in comparison to the measured sorption isotherm. The sorption isotherm has little impact but it remains an essential factor for deriving the $a_{w,s}$ from the water content calculated by the water transfer model.

4.3.2. Representativeness of the validation experiment

Product thickness can affect the prediction of $a_{w,s}$ because the thicker the product, the higher the available amount of water. The thickness varied from 0.5 to 5 cm. Under test conditions, Fig. 6 shows that an increase in the thickness exceeding 1.5 cm has no effect on either the evolution of $a_{w,s}$ and the bacterial growth. In other words, the observations would have been similar on larger pieces of meat.

4.4. Analysis of the combined effect of air velocity and RH

In the food industry, air velocity in contact with foods varies in the range of 0.2–5 m/s. Low air velocities are mainly encountered in chilled rooms for storage or in ageing rooms and high ones are generally found in thermal apparatus like chilling tunnels.

In a first set of calculations, the air velocity varied along the whole range while the RH was that of the validation experiment. Fig. 7 shows the dramatic incidence of this variable. At 0.2 m/s, the $a_{w,s}$ decreases slowly until it reaches 0.99 for 14 days, and the bacterial growth rate is close to the maximum that is close to that of the Petri dish experiment. On the other hand, bacterial growth is severely limited at an air velocity equal to 5 m/s.

In a chilled room, the RH is not well controlled and the air velocity greatly varies from place to place depending on the location of fans (Mirade & Picgirard, 2001). Thus,

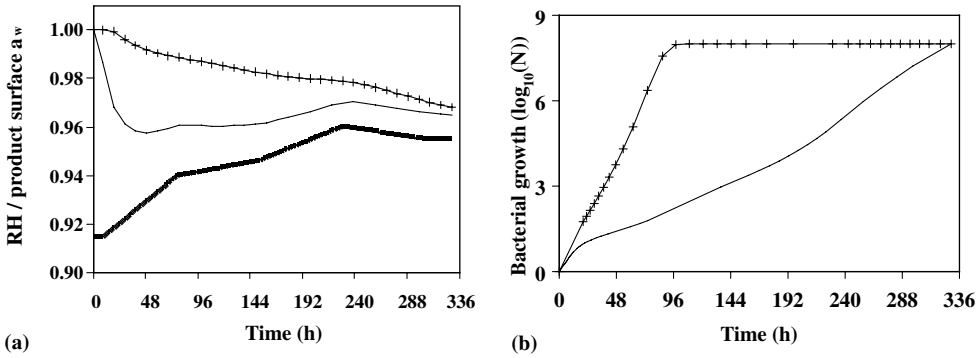

Fig. 4. Effect of water diffusivity [$D=f(X)$(—), $D=2\times10^{-10}$ (—+—)] at 12 °C (a) on the evolution of the a_w at the surface of pork meat and (b) on the bacterial growth. RH time variation of the validation experiment (—) was used for the calculations.

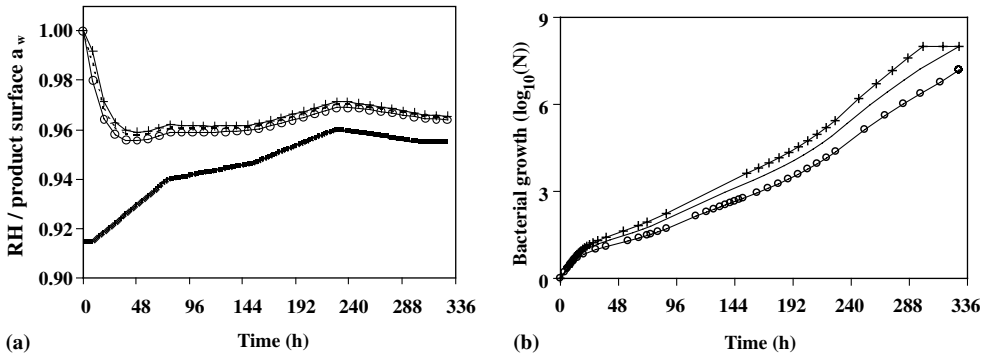

Fig. 5. Effect of the sorption curve [Reference (---), −20% (—+—), +20% (-·-)] (a) on the evolution of the a_w at the surface of pork meat and (b) on the bacterial growth. RH time variation of the validation experiment (—) was used for the calculations.

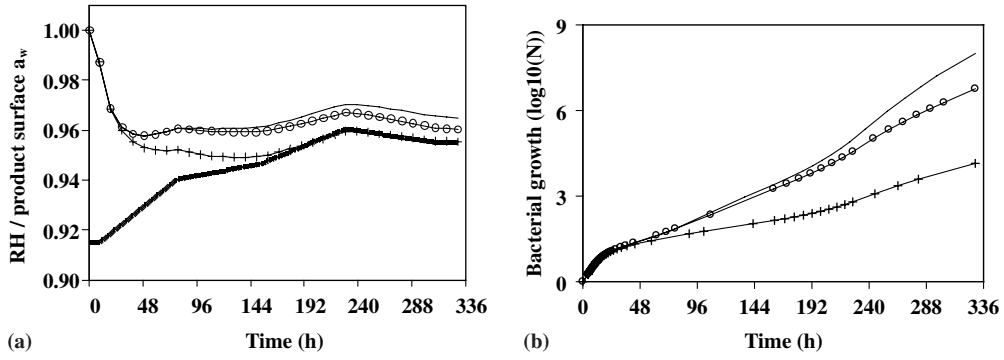

Fig. 6. Effect of the product width [0.5 cm(—+—), 1.0 cm (-·-), 1.5 cm and above (—)] (a) on the evolution of the a_w on the surface of pork meat and (b) on the bacterial growth. RH time variation of the validation experiment (—) was used for the calculations.

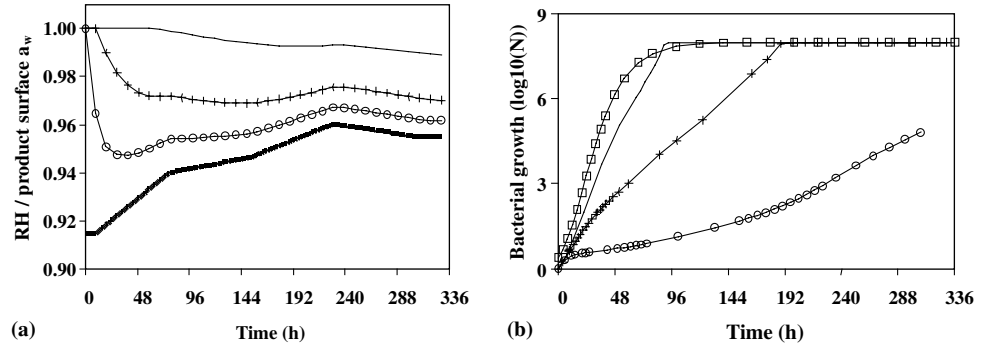

Fig. 7. Effect of air velocity [0.2 m/s (—), 1.0 m/s (—+—), 5.0 m/s (-·-)] (a) on the evolution of the a_w on the surface of pork meat (2 cm width) and (b) on the bacterial growth. RH time variation of the validation experiment (—) was used for the calculations. Comparison with the bacterial growth calculated in the Petri dish experiment where $a_w = 1$ (—□—).

a factorial experimental design was performed to study the impact of the two process parameters. Eleven levels of RH, ranging from 70% to 98%, and 10 levels of air velocity, from 0.1 to 1.0 m/s, were tested. Under each given condition, the increase in population of the *Pseudomonas* spp. was calculated after 96 h of storage at 12 °C. Fig. 8 outlines the huge impact of the two parameters on bacterial growth. With data like this, the conditions conducive or not conducive to *Pseudomonas* growth can be determined. If an increase of 2 log units is acceptable, Fig. 8 shows that when the air velocity is equal to 0.2 m/s, the RH must be maintained below 82%, which is quite dry, whereas it is possible for the RH to be as high as 90% if the air velocity is equal to 0.9 m/s.

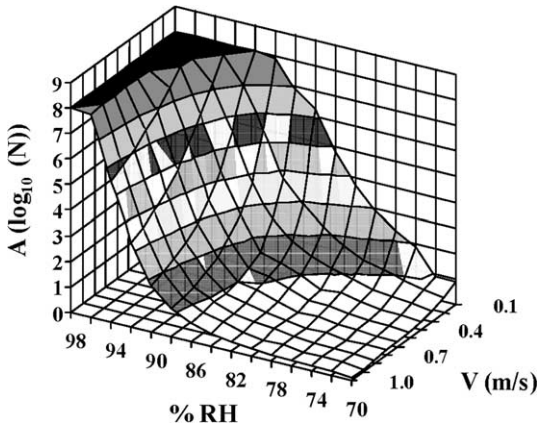

Fig. 8. Effect of relative humidity and air velocity on the *Pseudomonas* spp. population increase, A ($\log_{10}(N)$), on pork meat after 96 h. Initial contamination of *Pseudomonas* spp.: 10 CFU/cm^2. Ulog: log10(N) unit. $A \geqslant 8$ Ulog: ■; $7\,\text{Ulog} \leqslant A \leqslant 8\,\text{Ulog}$: ■; $6\,\text{Ulog} \leqslant A \leqslant 7\,\text{Ulog}$: ■; $5\,\text{Ulog} \leqslant A \leqslant 6\,\text{Ulog}$: ■; $4\,\text{Ulog} \leqslant A \leqslant 5\,\text{Ulog}$: □; $3\,\text{Ulog} \leqslant A \leqslant 4\,\text{Ulog}$: □; $2\,\text{Ulog} \leqslant A \leqslant 3\,\text{Ulog}$: □; $1\,\text{Ulog} \leqslant A \leqslant 2\,\text{Ulog}$: □; $0\,\text{Ulog} \leqslant A \leqslant 1\,\text{Ulog}$: □.

5. Conclusion

In this study, a water transfer model was developed and validated without any optimisation of the physical parameters. It was combined to a bacterial growth model and the whole model was tested to predict the growth of *Pseudomonas* spp. on the surface of pork meat subjected to air drying. Agreement between the calculation and the experimental results was observed with *Pseudomonas* spp. which have been chosen for their relevance as spoilage bacteria in pork. But *Pseudomonas* spp. were not so relevant to prove completely the validity of our approach. Indeed validation experiments have shown large variations of replicas in the bacterial determinations, indicating that the *Pseudomonas* population was very sensitive to changes in the surface a_w. This is mainly due to the fact that the minimum a_w for growth of this species is fairly high. Bacteria less sensitive to a_w should be studied to better validate the global model.

Nevertheless, the main advantages of integrating a water transfer model and a bacterial predictive model are that the effects of process parameters such as air temperature, velocity and relative humidity on the drying of a surface product can be compared and their indirect consequences on the bacterial growth quantified. Such an approach can be applied to other micro-organisms and can be particularly interesting for pathogenic bacteria for which many predictive models already exist (McMeekin & Ross, 1996).

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