Development and validation of a mathematical model for pseudomonads growth as a basis for predicting spoilage of fresh poultry breast and thigh fillets

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ABSTRACT The growth of naturally contaminated pseudomonads on fresh breast and thigh poultry fillets during aerobic storage was studied and modeled as a function of temperature $(0-30^{\circ}\text{C})$. A statistical comparison of the models for breast and thigh fillets showed that muscle type does not significantly affect the temperature dependence of pseudomonads growth kinetics. A unified model for breast and thigh was developed and validated against pseudomonads growth rate data under isothermal conditions extracted from literature and experimental data under dynamic temperature conditions. The validation results showed a satisfactory performance of the model with the bias and accuracy factors ranging from 0.85 to 1.09 and 1.02 to 1.21, respectively. The model was further used to predict the shelf life of fresh poultry as the time required by pseudomonads to reach the spoilage level for various scenarios of temperature, initial contamination level, and physiological state of pseudomonads demonstrating its application in a risk-based shelf-life assessment of fresh poultry products.

Key words: Pseudomonas spp., poultry fillet, bacterial growth, spoilage, predictive microbiology

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INTRODUCTION

Food spoilage constitutes a significant financial burden for the food industry with considerable economic losses (FAO, 2011). In addition, the spoilage of foods contributes greatly to the total food losses and wastage, one of the most important problems of the modern world, since approximately one-third of all food that is suitable for human consumption, is lost or wasted globally. The Food Waste Index report presented by the United Nations showed that 931 million tons of edible food are annually wasted worldwide consisting about the 17% of the total food that was available to consumers in 2019 (United Nations Environment Programme, 2021). This equates to almost \$940 billion in economic losses each year, while one in 9 people is still malnourished. Specifically, 40% of food is lost or wasted, costing an estimated \$218 billion only in the United

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States (United States Environmental Protection, 2022). Furthermore, food loss and waste also result in significant environmental impact being responsible for the 8% of the world's greenhouse gas emissions (FAO, 2015). Therefore, the control of food spoilage is a great challenge for reducing economic losses and improves efficiency within food businesses, redirecting food to those who need it and reducing environmental impacts of food waste.

Food spoilage is described as the process of alterations in the sensory characteristics of a food product that renders it unacceptable to the consumer. Such alterations may include physical damage, chemical changes (e.g., oxidation), off-flavors, and off-odors or changes in appearance (e.g., visual microbial growth, color changes). Microbial growth and metabolic activity are by far the most common causes of spoilage and may be manifested as visible growth (e.g., slime, fungal mycelia), as textural changes (degradation of polymers, coagulation), or as off-odors and off-flavors following microbial metabolic activity depending on various intrinsic, extrinsic, implicit, and processing factors (Nychas and Tassou, 1997; Tsigarida and Nychas, 2001; Gram and Dalgaard, 2002).

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Among the various foodstuffs, fresh poultry is probably the most perishable and highly susceptible to microbiological spoilage mainly due to its nutrient composition and the high pH and water content which allow fast growth of spoilage bacteria. Under aerobic conditions, spoilage of fresh poultry is caused by pseudomonads which have been characterized as the "Specific Spoilage Organisms" (SSO) of most of the aerobically packed fresh meat products (Koutsoumanis et al., 2006; Doulgeraki et al., 2012; Saenz-García et al., 2020). Growth of pseudomonads on poultry meat results in the production of metabolic products such total volatile basic nitrogen (TVB-N) leading to off-odors and sensory rejection when their level reaches a spoilage level (SL) about 10^7 cfu/g (Koutsoumanis et al., 2006; Zhang et al., 2012).

Temperature is the most important environmental factor affecting growth of pseudomonads during distribution and storage (Koutsoumanis et al., 2006; Dominguez and Schaffner, 2007; Gospavic et al., 2008) and even short-temperature deviations may result in significantly shorter times to spoilage (Moore and Sheldon, 2003). Other factors that may affect pseudomonads growth include the composition and the pH of poultry meat. Indeed, differences in the above factors between carcass parts such as breast and thigh (Huda et al., 2011; Chen et al., 2016) may result in respective differences on microbial growth and the spoilage potential (Dourou et al., 2021).

Controlling poultry spoilage requires an effective expiration date (use-by date) which leads to the maximum exploitation of the "true" product's shelf life minimizing the risk of while spoilage (Koutsoumanis et al., 2021). Assessment of expiration date of foods is traditionally carried out in challenge tests, where all parameters are fixed, and the results are only applicable to the specific product and conditions tested. As an alternative, Predictive Food Microbiology (**PMF**) has the potential to predict, by interpolation, microbial growth, and food spoilage to conditions other than those experimentally tested and, thus, to overcome the limitations of challenge testing (McMeekin, 2007).

Based on the above, the objective of the present study was to develop and validate a microbial spoilage model for fresh poultry fillets based on pseudomonads growth. The growth behavior of naturally present pseudomonads was studied on both breast and thigh fillets in order to evaluate potential differences in the microbial kinetics. The growth kinetics data were modeled as a function of temperature and the developed model was validated at both static and dynamic storage temperature conditions. The model was further used to predict the shelf life of fresh poultry under various scenarios describing the variability and uncertainty of factors affecting spoilage such as the storage temperature, the initial contamination level and the physiological state of pseudomonads.

MATERIALS AND METHODS

Preparation of Poultry Samples

Chicken breast fillets (ca. 245-280 g per fillet) and chicken thigh fillets (ca. 90-110 g per fillet) were obtained from a Greek poultry industry and transported to the laboratory within 60 min under refrigeration. Each fillet was placed on a retail foam tray and overwrapped with air-permeable polyethylene plastic film and stored under controlled isothermal conditions (0, 5, 5)10, 15, 20, 25, and 30°C) in high precision (± 0.5 °C) incubation chambers (MIR-153, Sanyo Electric Co., Osaka, Japan). Two additional sets of packaged fillets were stored under 2 dynamic temperature profiles, namely: a) 12 h at 5°C, 8 h at 10°C and 4 h at 15°C; b) 12 h at 0°C, 8 h at 5°C and 4 h at 10°C. The temperature of samples was monitored during the storage period using electronic temperature monitoring devices (cox tracer, Cox Technologies, Belmont, NC). Duplicate packages for each storage temperature were taken at appropriate time intervals to allow for efficient kinetic analysis of microbial growth. Two independent experiments were conducted for each storage condition with duplicate samples analyzed per sampling point for isothermal storage conditions, whereas in the case of dynamic temperature profiles poultry samples were analyzed in triplicate.

Microbiological Analysis

Four slices with about 2-mm thickness of chicken breast and thigh fillets were aseptically removed using a sterile stainless steel cork borer (diameter: 2.5) cm), scalpel, and forceps. The contaminated surface (external fillet surface) of each slice was approximately 5 (4.91) cm². The four slices with a total contaminated surface of approximately 20 (19.64) cm², were added to 100 mL of sterile quarter strength Ringer's solution (Lab M Limited, Lancashire, UK) and homogenized in a Stomacher device (Lab Blender 400, Seward Medical, UK) for 120s at room temperature. About 0.1 mL of the appropriate decimal dilution was spread on Pseudomonas Agar Base supplemented with cephalothin-fucidin-cetrimide (LabM Limited) and incubated at 25°C for 48 h. After incubation, typical colonies of presumptive Pseudomonas spp. were enumerated, and the populations were expressed as $\log cfu/cm^2$. The selectivity of the medium was checked routinely by Gram staining and microscopic examination of smears prepared from randomly selected colonies.

Development of the Mathematical Model

The population data (log cfu/cm²) over time for each temperature were fitted to the primary model of Baranyi and Roberts (1994) using DMFit Excel add-in, in order to estimate the growth kinetic parameter (maximum specific growth rate, μ_{max}) and the physiological state (h_0) of the cells. The original dynamic model has

an explicit solution for static conditions (when the model parameters do not depend on time), which describes the natural logarithm of the cell concentration, $y(t) = \ln x$ (t), by the equation:

$$y(t) = y_0 + \mu_{\max}A(t) - \frac{1}{m}ln\left(1 + \frac{e^{m\mu_{\max}A(t)} - 1}{e^{m(y_{\max} - y_0)}}\right)$$
(1)

where μ_{max} is the maximum specific growth rate of the cell population; y_{max} is the natural logarithm of the maximum population's concentration; y_0 , the natural logarithm of the initial cell concentration; m is a curvature parameter characterizing the transition from the exponential to the stationary phase of growth and A(t) is a gradually delayed time variable described by the equation:

$$A(t) = t + \frac{1}{\mu_{\max}} ln \left(e^{-\mu_{\max}t} + e^{-h_0} + e^{-\mu_{\max}t - h_0} \right)$$
(2)

where h_0 is a parameter characterizing the 'adaptation work' required by the cells to adjust to the new environment (Baranyi and Roberts, 1994). The parameter h_0 is also related to the physiological state parameter α_0 $(h_0 = -\ln(\alpha_0))$.

The maximum specific growth rate was further modeled as a function of temperature using the square-root equation (Ratkowsky et al., 1982):

$$\sqrt{\mu_{\max}} = b \times (T - T_{\min}) \tag{3}$$

where b and T_{\min} are parameters to be estimated with T_{\min} representing the theoretical minimum temperature (°C) for pseudomonads growth.

At a first stage, 2 separate models (breast model [**BM**] and thigh model [**TM**]) were developed based on pseudomonads growth data on breast and thigh samples, respectively. The coefficients of the 2 models were compared with regression analysis using Minitab 17 (Minitab, 2010) to check if their constants and slope coefficients are statistically different. At a second stage a unified model (**UM**) was developed based on pseudomonads growth data on both breast and thigh samples.

Model Validation

For the validation of the developed unified model, a literature search was conducted, in which 5 published studies were identified and used as sources for collecting *Pseudomonas* spp. maximum growth rate values on different fresh poultry products stored at various temperature isothermal conditions from 0 to 25°C Moore and Sheldon, 2003; Dominguez and Schaffner, 2007; Gospavic et al., 2008; Raab et al., 2008; Galarz, et al., 2016). The growth rates predicted by the developed unified model were compared with those extracted from the literature. Additionally, the developed unified model was validated at non-isothermal conditions by comparing predictions with the observed growth of pseudomonads on breast and thigh fillets in experiments at 2 different dynamic temperature profiles performed in the present study. In the latter case the growth of pseudomonads at non-isothermal conditions was predicted by combining the square root secondary model (Eq. (3)) with primary model (Eqs. (1) and ((2))). The prediction of growth at dynamic temperature conditions was based on the assumption that the growth rate is adapted instantaneously to the new environment after a temperature change (Koutsoumanis et al., 2006).

The performance of the developed model was evaluated by a graphical comparison between the observed and the predictive growth and by estimating the bias (B_f) and accuracy (A_f) factors as proposed by Ross (1996):

$$B_f = 10^{\left(\sum_{i=1}^n \log(pd_i/ob_i)/n\right)}$$
(4)

$$\mathbf{A}_{f} = 10^{\left(\sum_{i=1}^{n} \left| \log(pd_{i}/ob_{i} \right|/n)\right)}$$
(5)

For the validation against literature data, pd_i and ob_i are the predicted and observed growth rates, respectively. For the validation at dynamic temperature conditions where the growth rate is not constant, the bias and accuracy factors were calculated based on the population level. In the latter case ob_i is the observed population level at the experimental time, pd_i is the population level predicted by the model at the same time and n is the number of observations.

RESULTS AND DISCUSSION

Model Development

Figure 1 shows the observed growth data of pseudomonads on fresh breast and thigh fillets during storage at the different tested isothermal temperature conditions. The growth data of each replicate were fitted to the Baranyi and Roberts (1994) model to estimate the growth kinetic parameters (Figure 2). Overall, the fitting of the growth data to the primary model was satisfactory with an average regression coefficient (\mathbf{R}^2) of 0.96 for chicken breast and 0.91 for chicken thigh. The maximum specific growth rate of pseudomonads on breast fillet increased from $0.023 \pm 0.003 \text{ h}^{-1}$ at 0° to $0.65 \pm 0.058 \text{ h}^{-1}$ at 30°C. The rates were $0.034 \pm 0.005 \text{ h}^{-1}$ and $0.72 \pm 0.049 \text{ h}^{-1}$ for thigh fillets stored at 0 and 30°C, respectively. In most cases pseudomonads grew without a lag phase. In particular, no lag phase was observed for pseudomonads growth in all 28 replicate experiments with thigh fillets stored at the various storage temperatures. For experiments with breast fillets a lag phase was observed in 6 of the total 28 replicates with an average value for the physiological state parameter α_0 of 0.87. The above kinetics are similar to those reported for pseudomonads in raw poultry in previous studies (Moore and Sheldon, 2003; Dominguez and Schaffner, 2007; Gospavic et al., 2008). However, no study is available in the literature presenting a systematic comparison between growth kinetics of pseudomonads on different parts of poultry carcasses such as



(B)

Figure 1. Growth data of *Pseudomonas* spp. in chicken breast (A) and chicken thigh (B) during storage at different temperature conditions. Error bars indicate the standard error between experimental replicates.

breast or thigh. Thus, a question that arises is whether the type of poultry muscle can be an important factor in a predictive spoilage model for raw poultry products.

To address the above question, separate models were developed in the present study for the effect of storage temperature on the growth of pseudomonads on breast and thigh fillets using the square root Ratkowsky equation (Ratkowsky et al., 1982). Figure 3 shows a graphical representation of the observed data and the fitted regression lines of the breast model (**BM**) and the thigh model (**TM**). The estimated models' parameters and the statistics of the fittings are presented in Table 1.



Figure 2. Representative *Pseudomonas* spp. growth data fitted to the Baranyi and Roberts (1994) model for chicken breast (A–C) and thigh (D-F) stored at 10° (A, D), 15° (B, E) and 25°C (C, F). Points (\diamond) represent the observed values (log cfu/cm²), and line (–) represents the fitting.



Figure 3. Effect of temperature on the maximum specific growth rate (μ_{max}) of *Pseudomonas* spp. on chicken breast (- - -) and chicken thigh (—) fitted to the square root model (Eq. 3). Points (\square and \bigcirc for chicken breast and thigh, respectively) represent the observed values of μ_{max} .

Table 1. Estimated parameters of the square root models forchicken breast and thigh.

	Estimated value	$\begin{array}{c} \text{Lower} \\ 95\% \text{CL}^1 \end{array}$	$\begin{array}{c} {\rm Upper} \\ {\rm 95\% \ CL} \end{array}$	R^2
Chicken b	reast model			
b	0.0218	0.021	0.023	0.983
T_{min}	-5.83	-7.03	-4.63	
Chicken th	nigh model			
b	0.0226	0.021	0.024	0.967
T_{min}	-6.60	-8.36	-4.83	

¹Abbreviation: CL, confidence level.

The estimated theoretical minimum temperatures for growth $(T_{\rm min})$ were -5.83°C and -6.60°C for the breast and the thigh model, respectively. For the slope parameter b the estimated values were 0.0218 and 0.0226, respectively. A comparison between the 2 models showed no statistical differences for both intercept and slope parameters (Table 2).

Breast and thigh poultry muscle differ in the physicochemical and nutritional properties regardless of chicken breeds (Chen et al., 2016). Breast muscle pH is in general lower than that of the thigh since the former has been shown to contain higher amounts of glycogen, and therefore, lactic acid (Nychas and Board. 1991:Kakouri and Nychas, 1994).Dourou et al. (2021) reported pH values of 6.05 and 6.56 for fresh chicken breast and thigh, respectively. Chen et al. (2016) also showed that the protein content in chicken breast is significantly higher than that of the thigh while the intramuscular fat content is double in thigh compared to breast in chicken and duck raw cuts (Huda et al., 2011; Chen et al., 2016). The results of the present study showed that the

 Table 2. Regression comparison of the square root models for chicken breast and thigh.

Term	Coefficient	Standard error	P-value
A^1	-0.0230	0.0176	0.196
B^2	-0.000643	0.000977	0.513

¹A refers to the difference between the two slopes.

²B refers to the difference between the two constants.



Figure 4. Effect of temperature on the maximum specific growth rate ($\mu_{\rm max}$) of *Pseudomonas* spp. on both chicken breast and thigh fitted (-) to the square root model (Eq. 3). Dashed (- -) lines indicate the 95% prediction limits. Red points (•) represent the observed values of $\mu_{\rm max}$. Blue points (\odot) represent literature data collected from previous studies.

above differences between breast and thigh muscle do not significantly affect the growth potential of pseudomonads neither the temperature dependence of the growth kinetics. According to this observation a unified model was developed based on the growth rate data from both breast and thigh experiments (Figure 4). Data fitting provided an $R^2 = 0.970$ (Table 3) indicating that the square root model described satisfactorily the effect of temperature on the growth rate of pseudomonads on breast and thigh poultry meat. The estimated theoretical minimum temperature for growth ($T_{\rm min}$) and the slope parameter b were -6.18°C and 0.022, respectively (Table 3).

Model Validation

The developed unified model was further validated against pseudomonads growth rate data under isothermal conditions extracted from literature and experimental data under dynamic temperature conditions produced in the present study. Forty-two (42) growth rates of *Pseudomonas* spp. were retrieved from the literature referring to various chicken products (fillets, drumsticks, thighs) stored under aerobic conditions at various temperatures ranging from 0 to 25°C (Moore and Sheldon, 2003; Dominguez and Schaffner, 2007; Gospavic et al., 2008; Raab et al., 2008; Galarz, et al., 2016). A graphical comparison between predicted growth rates by the unified model and observed growth rates from literature studies is also presented in Figure 4. As shown in the latter figure 30 of the total 42 growth rates from literature fall within the 95% prediction intervals of the developed unified model. The calculated bias and accuracy factors were 0.85 and 1.21, respectively (Table 4). The performance of a model developed for spoilage bacteria is considered acceptable when the B_f is lower than 1.25 (Dalgaard, 2000). The overall B_f demonstrates that the model can satisfactorily predict the growth of pseudomonads (Mellefont et al.,

 Table 3. Estimated parameters of the square root unified model.

	Estimated value	Lower 95% CL	Upper 95% CL	R^2
Unified n	nodel			
b	0.022	0.021	0.023	0.970
T_{min}	-6.18	-7.32	-5.04	

Table 4. Estimated bias (B_f) and accuracy (A_f) factors for the developed unified model against literature isothermal data and experimental data on two dynamic temperature profiles.

		B_f	A_f
Literature data		0.85	1.21
Experimental data/product	Storage	B_f	A_f
Breast	Profile a Profile b Both profiles Profile a	$1.09 \\ 1.01 \\ 1.05 \\ 1.05$	$1.12 \\ 1.02 \\ 1.06 \\ 1.06$
Overall	Profile b Both profiles	1.06 1.05	1.10 1.08 1.07

2003; Longhi et al., 2013). Concerning the A_f , Mellefont and colleagues suggest that an A_f equal to 1.3 is considered acceptable (Mellefont et al., 2003). Hence, both the graphical evaluation and the B_f and A_f factors demonstrated a satisfactory performance of the model in predicting the growth of pseudomonads in poultry products stored under isothermal conditions.

The developed unified model was also validated against observed pseudomonads growth on both chicken breast and thigh, stored under 2 non-isothermal temperature scenarios in order to evaluate its performance under more realistic chill chain conditions. The growth of pseudomonads under dynamic temperature conditions was predicted assuming $\alpha_0 = 1$ (no lag phase) and a maximum population concentration $N_{\rm max}=10^7~{\rm cfu}/$ cm^2 based on the experiments at isothermal conditions. A graphical comparison between the observed and predicted growth of pseudomonads stored at dynamic temperature conditions is presented in Figure 5. Overall, the predictions of the unified model were in accordance with the observed growth indicating that the assumption that the growth rate is adopted instantaneously to the new environment after a temperature change is valid.

In addition to graphical comparison, the performance of the unified model was assessed using the bias and accuracy factors (Table 4). Based on the growth data from the 2 tested profiles, B_f and A_f factors were estimated for each profile and chicken product. As presented in Table 4, the bias factor ranged between 1.01 and 1.09 while the accuracy factor ranged between 1.02 and 1.12 among the tested temperature profiles. The overall B_f and A_f for all profiles were 1.05 and 1.07 showing a satisfactory performance of the model. B_f and A_f were also estimated for the independent secondary models for breast and thigh. B_f and A_f were 1.04 and 1.05 for breast model and 1.08 and 1.08 for thigh model respectively, confirming that the unified model is equivalent with the independent secondary models for breast and thigh.



Figure 5. Comparison between observed (\blacksquare) and predicted growth (-) of *Pseudomonas* spp in chicken breast (A and B) and chicken thigh (C and D) stored under non-isothermal conditions (A, C: 12 h at 5°C, 8 h at 10°C and 4 h at 15°C and B, D: 12 h at 0°C, 8 h at 5°C and 4 h at 10°C). Red dashed (- -) lines indicate the 95% prediction limits. Blue dashed dot (- • -) lines indicate the temperature profiles.

Application of the Pseudomonads Growth Model to Predict the Shelf Life of Fresh Poultry

Microbiological spoilage of aerobically stored fresh poultry is caused by pseudomonads which have been characterized as the "SSO" for this group of products. Growth of pseudomonads to a spoilage level of approximately 10^7 cfu/g or cfu/cm² results to organoleptic rejection due to off-flavor and off-odor. Shelf life (or Time-to-Spoilage **[TTS]**) of a fresh poultry product can be assessed using a predictive model as the time required by the pseudomonads to reach the spoilage level. Based on the above, the shelf life of fresh poultry depends on the factors affecting the time required by pseudomonads to multiply from their initial level to the spoilage level. These factors include mainly the initial contamination level of pseudomonads at the time of packaging, the extend of pseudomonads lag phase and the storage temperature. The developed unified model for pseudomonads growth was used to evaluate the impact of the above factors on the shelf life of fresh poultry. Figure 6 presents the effect of storage temperature on the shelf of poultry products with different initial life

contamination levels and physiological state parameters of pseudomonads. Based on the prediction of the unified model the shelf life of fresh poultry with an initial pseudomonads level of 2 log cfu/cm² decreases from 25.2 d at 0°C to 9.3 d at 4°C and 4.8 d at 8°C. Accordingly, for a fresh poultry product stored at 4°C the shelf life decreases from 9.3 d to 7.5 and 5.6 d for initial pseudomonads levels of 2, 3, and 4 log cfu/cm², respectively (Figure 6A). In relation to the impact of the physiological state and the lag phase of pseudomonads, for a product with an initial pseudomonads level of 2 log cfu/cm² minitial pseudomonads level of 1 (no lag phase) to the values of 0.5 and 0.1 results in a respective increase of shelf life from 9.3 d to 10.6 and 13.7 d (Figure 6B).

The developed unified model can be used as the basis for an effective shelf life assessment encompassing knowledge of the above factors and their interactions that affect the number of spoilage pseudomonads in fresh poultry products at the time of package opening by the consumer and their impact on spoilage. Koutsoumanis et al. (2021) stressed the need for taking into account the uncertainty and variability of factors affecting spoilage using Quantitative Microbiological



(A)



(B)

Figure 6. Predicted shelf life for fresh chicken products during storage at various temperatures with (A) representative levels of initial contamination (N₀: 2,3 and 4 log cfu/cm²) and $\alpha_0 = 1$, and (B) representative physiological state levels ($\alpha_0 = 1$, $\alpha_0 = 0.5$, $\alpha_0 = 0.1$) and initial contamination 2 log cfu/cm².

Risk Assessment (QMRA) methodology. The state of the art of microbial ecology and predictive microbiology allows for the development of probabilistic QMRA models for poultry spoilage that consider uncertainty and variability of factors affecting microbial behavior and spoilage. Such models can simulate what-if scenarios with different combinations of settings regarding product characteristics, process and storage conditions, expiration dating etc. of poultry products and assess their impact on the risk of spoilage. This can support the FBOs in selecting an effective expiration date (use-by or best before date), which leads to the maximum exploitation of the "true" product's shelf life, while minimizing the risk of spoilage to an acceptable (by the managers) level and providing the elements for a cost-benefit analogy in relation to the identified mitigation strategies for reducing the risk of spoilage and/or extending the shelf life of foods.

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DISCLOSURES

The authors have no conflicts of interest to declare.

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