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To cite this version:

M. Merai. Modelling of temperature, water activity and microbial growth on the surface of a pork leg during refrigerated transportation. FOODSIM’2018, Apr 2018, Louvain, Belgium. 5 p. hal-01872589

HAL Id: hal-01872589
https://hal.archives-ouvertes.fr/hal-01872589
Submitted on 12 Sep 2018

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MODELLING OF TEMPERATURE, WATER ACTIVITY AND MICROBIAL GROWTH ON THE SURFACE OF A PORK LEG DURING REFRIGERATED TRANSPORTATION

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KEYWORDS
3D model, pork leg, temperature, water activity, microbial growth, refrigerated transportation.

ABSTRACT
European regulation previously imposed to slaughterhouses that pork carcasses must be chilled immediately to ensure a core meat temperature below 7°C before future handling operation. Recently the regulation was amended and offers the opportunity to transport carcasses with a core temperature > 7°C from the slaughterhouse to the cutting plants but with continuous refrigeration during their transportation.

The purpose of this study was to evaluate the impact of the regulation modification on the microbial growth on the carcasses surface. First, the heat and mass transfer during refrigerated truck transportation were predicted using a 3D pork leg model. Predicted temperature and water activity of the carcasses surface were then used to evaluate microbial growth on the surface.

The obtained results showed that in the studied conditions (T_s = 4.5°C, HR = 97 %, T_i = 15°C), the leg reached the required temperature (< 7°C) at the core after 10 hours of transportation. Small change in water content/activity was observed. Based on the proposed predictive microbiology model, the maximum microbial load increase was 2.5 log_{10} after 30 hours of transportation.

NOMENCLATURE

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>k</td>
<td>mass (water) transfer coefficient (kg.m^{-2}.Pa^{-1}.s^{-1})</td>
</tr>
<tr>
<td>Le</td>
<td>Lewis number of air (Le = \frac{a_s}{D_a}=0.777)</td>
</tr>
<tr>
<td>L_v</td>
<td>latent heat of water evaporation (2450 kJ.kg^{-1})</td>
</tr>
<tr>
<td>M_w</td>
<td>molar mass of water (18 g.mol^{-1})</td>
</tr>
<tr>
<td>N</td>
<td>microbial load at the surface (CFU.cm^{-2})</td>
</tr>
<tr>
<td>P_{atm}</td>
<td>atmospheric pressure (101325 Pa)</td>
</tr>
<tr>
<td>P_{sat}</td>
<td>saturated vapor pressure (Pa)</td>
</tr>
<tr>
<td>q</td>
<td>heat flux (W.m^{-2})</td>
</tr>
<tr>
<td>RH</td>
<td>air relative humidity (%)</td>
</tr>
<tr>
<td>t</td>
<td>time of transportation (s)</td>
</tr>
<tr>
<td>T</td>
<td>leg temperature (K)</td>
</tr>
<tr>
<td>T_{min}</td>
<td>minimum temperature for microbial growth (K)</td>
</tr>
<tr>
<td>T_s</td>
<td>surface temperature at the muscle or rind part (K)</td>
</tr>
<tr>
<td>T_a</td>
<td>air temperature around the muscle or rind part (K)</td>
</tr>
<tr>
<td>X_{w}</td>
<td>water content in the leg (kg water/kg total)</td>
</tr>
<tr>
<td>Y</td>
<td>logarithmic microbial load at the surface log_{10}(N)</td>
</tr>
<tr>
<td>a_s</td>
<td>thermal diffusivity of air (m^2.s^{-1})</td>
</tr>
<tr>
<td>\mu</td>
<td>microbial growth rate (s^{-1})</td>
</tr>
<tr>
<td>\mu_{ref}</td>
<td>microbial reference growth rate (s^{-1})</td>
</tr>
<tr>
<td>\varphi_{water}</td>
<td>water evaporation flux from product surface (kg.m^{-2}.s^{-1})</td>
</tr>
<tr>
<td>\lambda</td>
<td>ham thermal conductivity (0.45 W.m^{-1}.K^{-1})</td>
</tr>
<tr>
<td>\rho</td>
<td>ham density (1072 kg.m^{-3})</td>
</tr>
</tbody>
</table>

INTRODUCTION
In order to prevent the pathogenic bacteria contamination in meat during slaughter operations, legislation requires the application of refrigeration immediately after post mortem inspection of carcasses. However, it is important to control the kinetics of product temperature reduction to optimize the meat maturation (ensuring a good technological quality) and the sanitary quality (Savell et al., 2005). French and European regulations have been enforced for the temperature of meat carcasses before and during transportation (Anonymous, 2004). According to this regulation, the meat must be chilled to reach a core temperature not exceeding 7°C as soon as possible. This refrigeration must be carried out in the
cold rooms of the slaughterhouse before any carcass handling operation such as transport or cutting. However, this strict regulation does not make the difference between the types of pathogenic and alterations microorganisms knowing that in the case of pork carcasses, there are essentially aerobic bacteria on the surface. Thus, in 2014, EFSA adopted a new scientific opinion which concluded that the surface temperature is an appropriate indicator of bacterial growth. Consequently, derogations have been adopted in some countries to allow the transport of carcasses or half-carcasses with a core temperature above 7°C, if the transport duration is less than 2 hours.

These derogations eventually triggered in 2016 a new scientific opinion on the growth of bacteria during the storage and transport of meat carcasses (EFSA, 2016). As some bacteria, especially Pseudomonas spp., can reach critical levels much faster than pathogenic bacteria, their growth kinetics may be an indicator of the temperature abuse during storage and transport.

Because of the air temperature and velocity heterogeneity in a refrigerated semitrailer, lower product temperature is often observed at the front and higher at the rear. The position of sanitary risk (zone of high temperature and low velocity) can be influenced by the semitrailer design and the carcass arrangement. Thus, an aerodynamic and thermal study in a refrigerated semitrailer loaded with meat carcasses is essential for the understanding of the airflow and the heat transfer coefficient variation at different position (Merai et al., 2018). This allows the identification of risk zones (areas where low air velocities and low convective transfer coefficients are observed).

The purpose of this study was to evaluate the impact of the regulation modification on the microbial growth on the carcasses surface. First, the heat and mass transfer during refrigerated truck transportation were predicted using a 3D pork leg model. Predicted temperature and water activity of the carcasses surface were then used to evaluate microbial growth on the surface. The developed model can be used in the future to study the influence of transport conditions (e.g. loading temperature of carcasses and air relative humidity) on the growth of bacteria at different areas in refrigerated semitrailer.

MATERIALS AND METHODS

Our scientific problematic is the simultaneous heat transfer, mass (water) transfer and microbial growth in a complex geometry object such as a meat carcass exposed to different operating conditions encountered in a refrigerated semitrailer. In this case, several transport phenomena occur, heat transfer by conduction inside the product and convection between product surface and air. Water migrates from the product inside to the surface and evaporates into the surrounding air. The product temperature and the water activity at the surface are determining factors of microbial growth.

3D geometry and meshing

A 3D model developed in COMSOL ® Multiphysics by Harkouss et al., 2018 of a pork leg was used in order to predict the temperature, water activity and microbial growth evolution on the surface of muscle part during transportation (collaboration with INRA-Theix, France).

Figure 1: Views of (a) the 3D leg geometry and its dimensions and (b) the volumetric tetrahedral mesh with the different components of the leg imported into Comsol ® Multiphysics software (c) two considered zones on the leg (Harkouss et al., 2018).
Based on X-ray imaging of a real pork leg, a 3D geometry was produced respecting its different parts, i.e. rind, muscles and bone (Figure 1a). A volumetric mesh was then constructed on the 3D geometry (42 cm of length; 32 cm of width and 15.8 cm of depth) (Figure 1b) consisting in 202000 tetrahedral meshes and containing the different parts. Two zones were considered: rind part with low water evaporation and muscle part with water evaporation (Figure 1c).

**Governing equations**

**Heat transfer modelling**

In order to numerically simulate heat transfer inside the leg and predict temperature evolution during transportation, Fourier law was used:

\[ \rho C_p \frac{dT}{dt} = \nabla \cdot (\lambda \nabla T) \] (1)

This equation was applied and solved in all domains of the numerical leg, except the bone which was considered as thermally insulated.

At the air-leg interface, both the thermal convection and the energy exchanged during water evaporation were taken into account. Thus, at this boundary the heat flow rate \( q \) was calculated as follow:

\[ q = -\lambda \nabla T \cdot \vec{n} = h(T_{\text{surface}} - T_{\text{air}}) + \varphi_{\text{water}} L_w \] (2)

**Mass transfer modelling**

The water migration inside the carcasses during transportation can be modelled by the Fick equation that was solved in all domains except in the bone:

\[ \frac{\partial C_w}{\partial t} = \nabla \cdot (D_w \nabla C_w) \] (3)

The water diffusion coefficient can be estimated by the equation presented by Ruiz-Cabrera et al. (2004) which is a function of water content \( X_{\text{water}} \):

\[ D_{\text{water}} = 4.10^{(0.625X_{\text{water}}-12)} \] (4)

For the boundary condition (product surface), water evaporation flux was calculated using the following equation:

\[ \varphi_w = -D_w \cdot \nabla C_w \cdot \vec{n} = k (a_w P_{\text{sat},T_a} - \frac{RH}{100} P_{\text{sat},T_a}) \] (5)

Water evaporation was assumed to occur only on the muscle part of the leg. \( k \) was calculated from the convective heat transfer coefficient using the Chilton Colburn analogy (Kondjoyan and Daudin, 1997):

\[ k = \frac{h M_{\text{water}}}{C_{p,\text{air}} M_{\text{air}} P_{\text{atm}} L_e^{2/3}} \] (6)

and \( P_{\text{sat},T_a} = \exp(-\frac{6764}{T_a} - 4.915 \cdot \log T_a + 58.75) \) (7)

Water activity was expressed as a function of water content (Rougier et al., 2007):

\[ a_w = 0.993 \exp(-0.0204 X_w^{-1.96}) \] (8)

Air temperature and relative humidity profiles were measured during real pork carcasses transportation. The convective heat transfer coefficient \( h \) was measured in a laboratory scale on the muscle part of the leg.

For the simulation, air temperature and relative humidity around the leg were considered constant \((\text{RH} = 97\%, T_a = 4.5^\circ C)\). Initial water content in the pork leg of 0.75 kg water/kg total and initial temperature of 15°C were assumed. Heat convective coefficient was fixed at 20 W.m\(^{-2}\).K\(^{-1}\).

**Microbial growth modelling**

In order to highlight the influence of surface temperature and water activity evolution on a possible microbial growth, a simple preliminary model without lag phase was used (eq.9).

\[ \frac{\partial Y(t)}{\partial t} = \mu (T, a_w) \] (9)

where \( \mu \) was obtained using a secondary model that takes into account temperature and water activity evolution:

\[ \mu = \mu_{\text{ref}} (T - T_{\text{min}})^2 \frac{a_w - a_{w,\text{min}}}{1 - a_{w,\text{min}}} \] (10)

No growth is assumed for \( T < T_{\text{min}} \) or \( a_w < a_{w,\text{min}} \). As a first approach, simulations were made for \( T_{\text{min}} = 4.5^\circ C, a_{w,\text{min}} = 0.9 \) and \( \mu_{\text{ref}} \) value is fixed at 0.003 s\(^{-1}\). These values depend on the microorganism and other food characteristics like salt concentration or pH. The \( T_{\text{min}} \) and \( a_{w,\text{min}} \) values were chosen as representatives of values that characterized psychrotrophic bacterial species involved in meat spoilage (Gill & Jones, 1992).

Supplementary studies are under way to estimate these values for specifics microorganisms often encountered on carcasses and meat cuts like *Pseudomonas*. 
RESULTS AND DISCUSSION

Temperature evolution during transportation

To visualize the temperature evolution inside the leg for 1 h, 5 h, 10 h and 30 h transportation, 4 horizontal slices have been selected using the same temperature scale (Figure 2).

Water activity evolution during transportation

Water activity varies slightly (results not shown) and can be considered constant around the initial value \(a_w \approx 0.96\). This small variation can be explained by the high relative humidity (97 %) of the air in the loaded refrigerated truck due to the air recycled during transportation.

Microbial growth during transportation

After 10 hours of transportation, order of magnitude of transportation time in France, microorganisms load increases and reaches \(\log_{10}\left(\frac{N}{N_0}\right) = 1.4\) (Figure 3). This value is consistent with observed increase of total psychrotropic microflora during carcass cooling (Gill and Jones, 1997; Afssa, 2009).

According to the European legislation (EFSA, 2016), for 30 hours transportation of carcasses with initial core temperature of 15°C, the contamination should not exceed \(10^4\) CFU.cm\(^{-2}\) (or \(4 \log_{10}\)). Predicted results showed that the logarithmic load increases: maximum value of \(\log_{10}\left(\frac{N}{N_0}\right)\) is lower than 2.5 after 30 hours (with \(T_i = 15^\circ C\)). To respect the regulation, the initial contamination should not exceed \(10^{1.5} \approx 300\) CFU.cm\(^{-2}\) in the studied conditions. This initial value of total microflora is consistent with existing literature (Gill and Jones, 1997).

Further work is to be conducted to evaluate the initial level of contamination on carcasses to accurately evaluate the final contamination.

Figure 2: Temperature distribution in four horizontal slices of the carcasses’ leg during transportation (t = 1 h, 5 h, 10 h and 30 h) showing the minimum and maximum temperature.

Figure 3: Microbial growth evolution during transportation (at 1 h, 5 h, 10 h and 30 h).
CONCLUSION AND PERSPECTIVES

Models were developed to predict the microbial growth on the surface of the lean part of a pork leg during transportation. This choice was based on the fact that the leg is the most problematic in the whole pork carcass as it is the largest part and so it is difficult to cool.

Temperature, water content and water activity evolution were simulated for 30 hours of transportation ($T_a = 4.5 \, ^\circ\text{C}$, $\text{RH} = 97\%$, $h = 20 \, \text{W.m}^{-2}.\text{K}^{-1}$, $T_0 = 15 \, ^\circ\text{C}$). Small changes were observed for water activity while temperature decreased rapidly at the surface of the leg ($< 7 \, ^\circ\text{C}$ after 1 hour). After 10 hours of transportation the microbial load increased and reached log$_{10} \left( \frac{N}{N_0} \right) = 1.4$.

In a future work, different scenarios will be studied varying the relative humidity, air temperature and the heat convection coefficient as a function of the position in the semi-trailer loaded with pork carcasses. This parametric study will allow a precise prediction of the microbial growth in the most problematic zones of the semi-trailer already identified in our previous work dealing with air velocity distribution around and between the carcasses.

ACKNOWLEDGEMENT

This research work was carried out within the framework of a DIM ASTREA grant by the Regional Council of Ile-de-France (France). The authors thank Pierre-Sylvain Mirade and Jason Sicard from INRA-Theix-France for their collaboration.

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