CHILLING OF CARCASSES FROM DOUBLE MUSCLED CATTLE : TIME-TEMPERATURE EVOLUTION AND PREDICTIVE MODELLING OF GROWTH OF *LISTERIA MONOCYTOGENES* AND *CLOSTRIDIUM PERFRINGENS*

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***Abstract* —The time/temperature combination during carcass chilling is of concern in order to avoid bacterial growth. The chilling speed is lower in carcasses with high muscular development such as large cattle from the Belgian Blue breed. Three slaughterhouses were selected for temperature and pH measurements during the chilling process at 6 different days on 4 half carcasses in order to obtain representative data from heavy carcasses with high muscular development. Predictive microbiology was used to evaluate the potential growth of *Listeria monocytogenes* and *Clostridium perfringens* on the surface and in the depth of the carcasses. The gamma concept was chosen as secondary model taking into account the effect of temperature, pH and water activity on the selected bacteria during the chilling process. The predicted growth potential of *Listeria monocytogenes* is influenced by the different environmental conditions of the selected slaughterhouses and could reach 1.4 log CFU/cm² after the chilling process. The potential growth of *Clostridium perfringens* is limited due to unfavourable conditions during the first hours and to low temperature later. It can be concluded that when the initial level of contaminating bacteria is not excessive the speed at which the carcass is currently chilled is sufficient to limit the growth of these two pathogens and to ensure the product quality.**

***Index Terms*—predictive microbiology, chilling, cattle, pathogens**

I. INTRODUCTION

According to the European Regulation (EC) N°853/2004 laying down specific hygiene rules for food of animal origin (Annex III, Chap. VII, point 1) (European Parliament and Council of the European Union, 2004), "*post-mortem inspection must be followed immediately by chilling in the slaughterhouse to ensure a temperature throughout the meat of not more than 3 °C for offal and 7 °C for other meat along a chilling curve that ensures a continuous decrease of the temperature*". This regulation does not mention any requirement for the speed and/or for the maximal duration of the chilling process. It is nevertheless well know that the chilling process cannot be too long, in order to avoid microbial growth, particularly in the depth of the carcass where putrefaction can occur quickly at high temperature, once anaerobic conditions have been reached. As an example, Rosset and Roussel-Ciquard (1984) recommend that an internal temperature of +15°C is reached in 24h *post-mortem*. Taking into account that the chilling speed is lower in heavy carcasses with high muscular development such as large cattle from the Belgian Blue (BB) breed, the first objective of the present experiment was to study the feasibility of such recommendations in large cattle from the BB breed, particularly in the centre of the hindquarter.

Predictive microbiology can be used to assess the risks of food processing, distribution, storage and food handling; and, to implement control measures in order to protect the microbiological quality, important for both food safety and product quality (Brul, van Gerwen & Zwietering, 2007). Predictive microbiology uses mathematical models (built with data from laboratory testing) and computer softwares to describe the responses of microorganisms to particular environmental conditions (McKellar, 2004). Thanks to predictive microbiology, the growth of selected bacteria can be simulated in function of temperature, pH and water activity (a_w) fluctuations. The second objective of the present experiment was to predict the potential growth of *Listeria monocytogenes* on the surface and *Clostridium perfringens* in the depth of carcasses from such animals.

II. MATERIALS AND METHODS

A. Data source of temperature and pH

Three slaughterhouses representative of practical chilling conditions usually observed in Belgium were selected for the present experiment, two of them (A, C) using a two steps chilling process (2h30 shock chilling + chilling room), the third one (B) using a 1 step chilling process (chilling room). In each slaughterhouse, the measurements were repeated at 6 different days on 4 half-carcasses corresponding to two different BB large cattle with the following characteristics : hot carcass weight : 494 ± 49, 473 ± 32 and 476 ± 25 kg in slaughterhouses A, B, and C respectively; European classification types DS2 or AS2. Temperature was registered continuously (1 measurement/min.) during 48h with Testo 171-4 or 171-8 data-loggers and thermocouple or thermistance probes (Testo, Lenzkirch, Germany) at three different locations : ambience near the carcass (measured at a height of 40 cm, between both half-carcasses distant of 10 cm),

superficially (under the superficial aponevrosis of the fore-leg, at the level of the *radial extensor of the carpus*, at half-distance between the bend and the distal end of the leg) and core (the deepest point of the hindquarter i.e. near the proximal end of the femoral bone, the probe being inserted via the interface between the *pectineus* and *adductor* muscles). The temperature usually being measured in the *longissimus dorsi* muscle, it was also punctually measured 1, 2, 4, 8 and 48h *post-mortem* at this location (at the level of the 7-8-9th ribs) in combination with pH, using a Knick Portamess type 913 Calimatic pH meter, a Mettler Toledo LoT406-M6-DXK combined insertion probe and a Pt1000 temperature probe.

B. Predictive microbiology

The growth of *Listeria monocytogenes* and *Clostridium perfringens* could be described by the linear three-phase primary growth model (Equation 1) (Buchanan, Whiting & Damert, 1997). The lag phase was neglected for *Listeria monocytogenes* (lag = 0 h). The lag phase of *Clostridium perfringens* was estimated at 10 h taking into account the positive redox potential period (Rosset & Roussel-Ciquard, 1984) and the time needed to germinate. During this period the anaerobic bacteria can not grow (International Commission on Microbiological Specifications for Foods, 1996). The a_w was estimated at 0.98 at the surface and 0.99 in the depth of the carcass (Anonymous, 2007). The initial concentration of *Listeria monocytogenes* at the surface and *Clostridium perfringens* in the depth of the carcass was fixed at 1.0 Log cfu/cm² and g, respectively, which can be considered as the "worst case". The gamma concept was chosen for secondary growth model to express the influence of temperature, pH and a_w on the growth rate (equations 2, 3, 4 and 5) (te Giffel & Zwietering, 1999).

The equations can be written as follow:

$$\begin{aligned}\ln(N_{t_k}) &= \ln(N_0) && \text{if } t \le lag \\ &= \ln(N_{t_{k-1}}) + \mu_{\max_{ij(k)}} \Delta t_k \\ &= \ln(N_{\max}) && \text{if } N(t) \ge N_{max}\end{aligned} \qquad \text{(Equation 1)}$$

$$\mu_{\max_{ij(k)}} = \mu_{opt}\gamma(T_{ij(k)})\gamma(pH_{ij(k)})\gamma(a_{w\,ij(k)}) \qquad \text{(Equation 2)}$$

$$\gamma(T_{ij(k)}) = \left[\frac{T_{ij(k)} - T_{\min}}{T_{opt} - T_{\min}}\right]^2 \qquad \text{(Equation 3)}$$

$$\gamma(pH_{ij(k)}) = \frac{(pH_{ij(k)} - pH_{\min})(pH_{\max} - pH_{ij(k)})}{(pH_{opt} - pH_{\min})(pH_{\max} - pH_{opt})} \qquad \text{(Equation 4)}$$

$$\gamma(a_{w_{ij(k)}}) = \frac{a_{w_{ij(k)}} - a_{w\min}}{1 - a_{w\min}} \qquad \text{(Equation 5)}$$

where:

i is one of the recorded steps along the chilling process with i = 1, ..., n
j is one of the three visited slaughterhouses with j = 1, 2, 3
k is the recorded parameter index in the stage i with k = 1,...,n_{ij}
Δt_k is the time interval with $\Delta t_k = t_k - t_{k-1}$. In the experiment, the Δt_k is constant and $\Delta t_k = \Delta t = 1$ hour
N_{t_k} is the bacterial population at time t_k (cfu.ml^{-1})
N_0 is the initial bacterial population (cfu.g^{-1} or cfu.cm^{-2})
μ_{opt} is the optimal bacterial growth rate (h^{-1})
$\mu_{\max_{ij(k)}}$ is the bacterial growth rate following the environmental factors at time t_k
$T_{ij(k)}$ is the recorded temperature at time t_k (°C)
$pH_{ij(k)}$ is the recorded pH at time t_k (°C)
$a_{w_{ij(k)}}$ is the recorded water activity at time t_k (°C)
$T_{\min}$ and T_{opt}, $pH_{\min}$ and pH_{opt}, $a_{w\min}$ and a_{wopt} are the theoretical minimal and optimal temperature, pH and water activity of growth for the considered bacterial strain, respectively (International Commission on Microbiological Specifications for Foods, 1996)

- for *Listeria monocytogenes* : $T_{\min}$ = -0.65°C, T_{opt} = 37°C, $pH_{\min}$ = 4.5, pH_{opt} = 6.7, $a_{w\min}$ = 0.92, a_{wopt} = 1
- for *Clostridium perfringens*: $T_{\min}$ = 9.8°C, T_{opt} = 43°C, $pH_{\min}$ = 5.0, pH_{opt} = 6.5, $a_{w\min}$ = 0.94, a_{wopt} = 1

To calculate μ_{opt} for each bacterial strain, the bacterial growth rate was collected from Combase by selection of the microorganisms with the specific environment.

III. RESULTS AND DISCUSSION

The superficial and core temperature evolutions of the carcasses are presented respectively in the figure 1 (a and b). The superficial temperature evolution was systematically in conformity with a recommendation from the Canadian Agency for Food Inspection (2002) suggesting that the temperature has to be lower than +7°C after 24h. This threshold has been reached after 12h, 9h and 11h on average in slaughterhouses A, B and C respectively. It is difficult to conclude about another recommendation from Rosset and Roussel-Ciquard (1984) who suggested that a temperature of +5°C is reached "as soon as possible". The internal temperature (+7°C) required by the European Regulation (EC) No 853/2004 has been reached around the 48th hour of refrigeration in the deepest point of the hind-leg of the carcasses evaluated during the present experiment. An internal temperature of +15°C within 24 hours *post-mortem* is recommended to reduce microbiological risks (Rosset & Roussel-Ciquard, 1984). This limit of +15°C was reached after 23 hours on average in the present study. Furthermore, according to the CSIRO Australian Division of Food Processing (1989), the +15°C threshold has to be reached after 20h. This recommendation seems to be difficult to put into practice with heavy BB carcasses. It has to be noticed that the temperature in the *longissimus dorsi* muscle cannot be used as an indicator of the "core temperature" since the temperature at that point was 3.6, 6.2, 13.3, 15.6 and 4.5°C lower than the deep leg temperature at 1h, 2h, 4h, 8h and 48h *post-mortem* respectively.

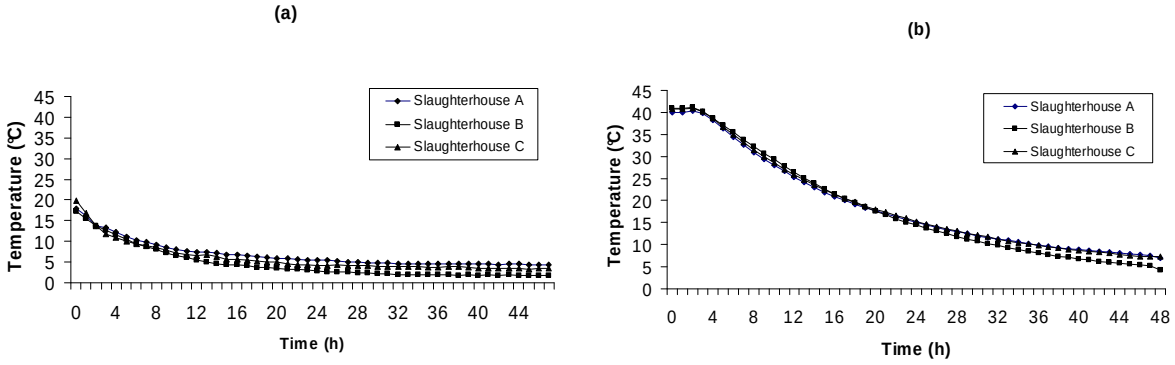

Figure 1 : Superficial (a) and core (b) temperature evolution of heavy half-carcasses from the Belgian Blue breed during the chilling process in three representative slaughterhouses.

The figure 2 gives the pH evolution in the *longissimus dorsi* muscle during chilling. For the slaughterhouses A and C, the average pH of the carcasses decreased from 6.6 to 5.8 during the first 8 h and further decreased slowly from 5.8 to 5.5 until the end of the process. In the slaughterhouse B, a faster evolution was observed due to electrical stimulation of the carcasses before chilling. The values of temperature in deep leg and in deep *longissimus dorsi* muscle combined with those of pH, led to the conclusion that in the three slaughterhouses, no risk of "*cold shortening*" (= alteration of tenderness due to an excessive chilling rate early *post-mortem*) has to be expected.

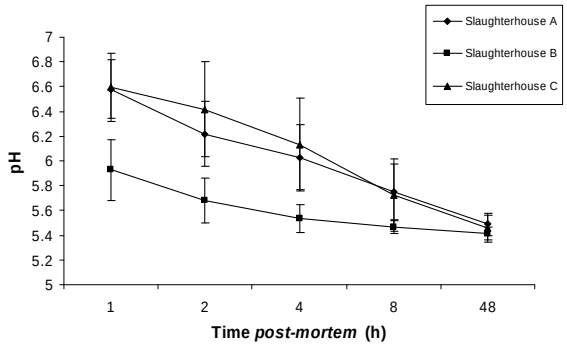

Figure 2 : *Post-mortem* evolution of pH in the *longissimus dorsi* muscle of heavy half-carcasses from the Belgian Blue breed during the chilling process in three representative slaughterhouses.

The figure 3 gives the predicted growth curves of *Listeria monocytogenes* (a) and *Clostridium perfringens* (b) in function of temperature and pH profiles measured in the three slaughterhouses. The growth potential during the chilling process for *Listeria monocytogenes* depends of the environmental conditions of the slaughterhouses and varies from 0.8 to 1.4 log cfu in function of the slaughterhouse ; the predicted growth being lower in the slaughterhouse B due to lower pH of the carcasses. The growth potential of *Listeria monocytogenes* has to be considered as important in the first hours of the chilling process where conditions are most favorable. Using electrical stimulation permits to reduce growth potential during the chilling process. The growth potential of *Clostridium perfringens* is similar for the three slaughterhouses and is estimated around 0.7 log cfu ; the effect of the observed pH variation being negligible. The positive redox potential during the first hours of the chilling process and the time needed to germinate prevents any

growth of this pathogen during this period. After this lag phase, the growth of *Clostridium perfringens* is limited due unfavorable temperature conditions, in particular after 16h and later when the internal temperature reaches values lower than +20°C.

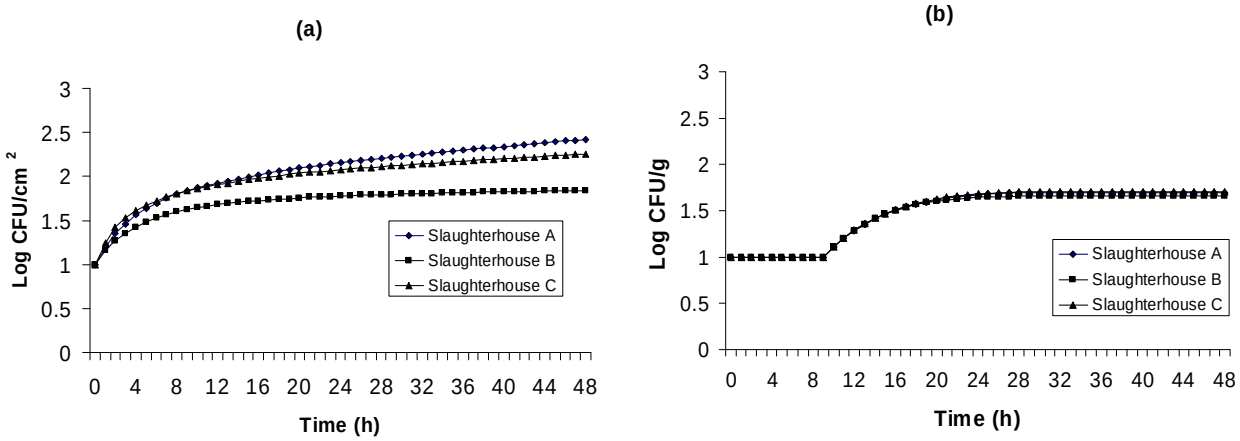

Figure 3: Predicted growth of *Listeria monocytogenes* on the surface (a) and *Clostridium perfringens* in the depth (b) of heavy half-carcasses from the Belgian Blue breed during the chilling process with the environmental conditions observed in three representative slaughterhouses.

IV. CONCLUSION

Control of the growth of pathogenic micro-organisms is the main food-safety concern during the chilling process of red-meat carcasses. The observed time-temperature combinations in the selected slaughterhouses comply with recommendations and limit the growth of these pathogens in case of accidental contamination of carcasses. This study contributes to demonstrate that modeling approach could be used on available environmental data in order to evaluate the risk associated with pathogens. The impact of the variation of environmental conditions can also be evaluated.

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