Probabilistic modeling of *Listeria monocytogenes* behaviour in diced bacon along the manufacture process chain.

E. Billoir[1], J.-B. Denis[1], N. Commeau[2,3], M. Cornu[2], and V. Zuliani[4]

[1] INRA, UR341 Mathématiques et informatique appliquées, F-78350 Jouy en Josas (elise.billoir@jouy.inra.fr)
[2] Afssa, Microbiologie quantitative et estimation des risques, 23 av. du Général de Gaulle, F-94706 Maisons-Alfort Cedex (m.simon-cornu@afssa.fr)
[3] UMR 518, Mathématiques et Informatique Appliquées, AgroParisTech/INRA, 16 rue Claude Bernard F-75005 Paris, France
[4] IFIP, Institut de la Filière Porcine, Pôle Viandes Fraîches et Produits Transformés, 7 av. du Général de Gaulle, F-94704 Maisons-Alfort Cedex (veronique.zuliani@ifip.asso.fr)

Abstract

To assess the impact of the manufacturing process on the fate of *L. monocytogenes*, we built a probabilistic model describing successively the different steps of the process. The model was actually designed as a hierarchical Bayesian network leading to the elicitation of human expertise. Contamination evolution was modelled in the adequate units (breasts, dices, then packaging units through the successive process steps). The use of probabilistic modeling allowed taking into account both the process intrinsic variability and parameter variability or uncertainty. Global statistics were deduced, diagrams showing the variability were drawn, and changes on the process were tested to look at the consequences on the final product.

Keywords

Process chain modeling; Bayesian network; *Listeria monocytogenes*; diced bacon;

Introduction

In France, 50% of the pork belly is transformed into diced bacon, and this production increases every year. The most recent data show that the *L. monocytogenes* prevalence for pork pieces decreased from 2001 to 2004 to reach about 25%. This level of prevalence remains however alarming when the pork pieces are used for the manufacture of raw meat products such as diced bacon. Indeed, during the diced bacon manufacturing, there is no physical or chemical treatment sufficiently drastic to eliminate the possibly present population of *L. monocytogenes*. Moreover diced bacon is occasionally consumed raw by 14% of French people (AFSSA, 2009).

In this work, we propose a basic model for the diced bacon process chain, from incoming breasts to outcoming packaging units, in order to evaluate the impact of each step on the contamination. Following the principles of Nauta's MPRM (2001), we adapted the model to the studied process, and to the specificity of the product, that is its solid and heterogeneity characteristics. Indeed, bacteria were assumed to be located only on the surface of the breasts, with a higher affinity for the lean areas than for the fat ones. By the mean of simulations, we exemplified the potential of the model. Simulations were performed with both a baseline calibration and alternative scenarios, in order to assess the impact of changes in the process and of accidental events.

Material and methods

Overview of the model

The model we built to study the behaviour of bacteria during the diced bacon manufacture process chain is a sequence of various steps: arrival, brining-and-tumbling, steaming, dicing and finally packaging. In the real process chain, storage steps occur between these steps. However, basing on reasonable assumptions concerning physico-chemical conditions and duration of the different storage steps, the bacterial contamination is expected not to evolve during them. Consequently we neglected the storage steps in the modeling, to focus on the other steps. During the process chain, the observation scale evolves, so we successively

followed the contamination of the breasts, the dices, then the packaging units. At every step, the contaminations were assumed to only depend on the state of the units at the end of the previous step and on modeling parameters. Thus we built our model as a Bayesian network. We introduced process intrinsic variability and, when available, parameter variability or uncertainty. In the diced bacon process chain, the batches considered at the different steps do not always match, they can be divided from one step to the following and they can overlap. For instance, a tumbling batch can be divided into several steaming batches and a dicing batch can gather several steaming batches. However in this study it was decided to follow-up the fate of tumbling batches of 1000 breasts, without overlaps later in the process.

Successive steps

(1) At **arrival**, *L. monocytogenes* contamination (Colony Forming Unit) on every breast was assumed to follow a conditional Poisson distribution of parameter equal to the breast concentration times the breast mass. (2) During the **tumbling-and-brining** step the breasts gathered in a batch were assumed to swap bacteria during the tumbling step. A part of the bacteria were assumed to be released from the breasts during this step. Then some bacteria were assumed to be lost because of staying hanging on the tumbler surface, whereas the others were assumed to be reallocated on the breasts of the tumbling batch. These two events (release and loss) were simulated by the mean of binomial drawings. Finally, the reallocated bacteria were distributed to the batch breasts using a multinomial distribution, in order to ensure the conservation of bacteria. Besides, the breasts were supposed to absorb the brine, so that their mass slightly increased. (3) To model the **steaming** step we used classical deterministic predictive microbiology tools for inactivation. (4) For the **dicing** step, we had to estimate how many dices would result from each breast. We began with geometrical considerations, assuming that every breast had a parallelepiped shape with dimensions proportional to the cubic root of their volume. The bacteria of each breast were shared between the resulting dices by a multinomial drawing, with allocation probability proportional to the exterior area, weighted by a fat/lean affinity ratio. Indeed, the bacteria are known to have a lower affinity with fat areas than with lean areas and we considered that one of the main faces of the breast was fat. Also we assumed that an additional contamination can come from the dicing machine, where bacteria can grow in some surfaces inaccessible for cleaning. (5) To model the **packaging** step, we cumulated the dice contaminations (CFU) by packaging units.

Baseline calibration of the model

To calibrate the model, the parameter estimation was carried out (i) from data given by partner business operators for the contamination at arrival and for the process parameters, (ii) from data given by IFIP for the geometrical parameters, (iii) from the literature for the inactivation parameters or (iv) basing on expert's opinions.

Alternative scenarios

In addition to the baseline calibration, we tested the following alternative scenarios: (S1) packaging units of 200g instead of 100g in the baseline, (S2) dice section of 5 mm instead of 8 mm in the baseline, (S3) steaming at an equivalent temperature of 50°C instead of 45°C in the baseline, (S4) initial contamination ten times higher than baseline, and (S5) initial contamination one hundred times higher than baseline.

Results and discussion

Results

As an example, Figure 1 shows the results of one simulation performed using the baseline calibration. The contamination distribution after brining-and-tumbling was narrower than after arrival. During the brining-and-tumbling step, the contamination was homogenized within the breasts. Then steaming reduced the contamination. This simulation predicted that a large proportion of dices were not contaminated by *L. monocytogenes*. In the packaging units,

L. monocytogenes prevalence was not negligible, however the contamination level remained low.

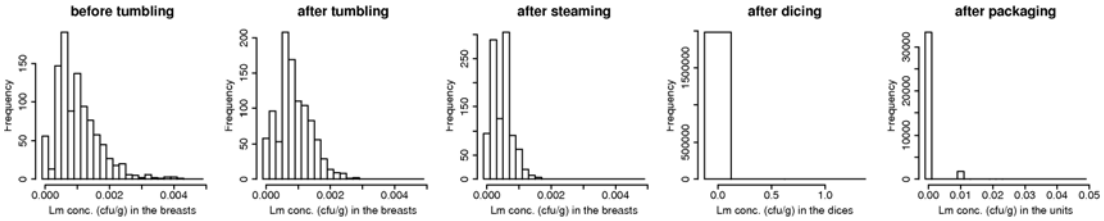

Figure 1: Results of one simulation. Bacterial concentration (CFU/g) after the five steps considered. First three graphs: on breasts, fourth graph: on dices, fifth graph: in packaging units.

Looking at the result of one simulation allows to qualitatively comment on every step. However, because our model is probabilistic, every simulation is different and numerous simulations were necessary to describe the baseline model outputs. According to the proposed model and its baseline calibration, every tumbling batch of 1000 breasts lead to 1 986 000 [1 976 000-1 996 000] dices and then 35 910 [35 610-36 210] packaging units. Figure 2 shows the results of 10,000 simulations. For each process step, the median simulation results is plotted (solid lines), as well as the 2.5% and 97.5% quantiles of the simulation results (short-dashed lines), corresponding respectively to low and high contamination levels. At every step the simulation results were quite close, indicating a low variability between simulations. In the median simulation results, at arrival about 5% of the breasts were uncontaminated by *L. monocytogenes*, 50% of the breasts had no more than 0.001 CFU/g, and 100% of the breasts had no more than about 0.005 CFU/g. After brining-and-tumbling, 100% of the breasts had no more than about 0.003 CFU/g. The steaming step reduced *L. monocytogenes* contamination so that 50% of the breasts had no more than 0.0005 CFU/g (that is 1 CFU on a breast), and 100% of the breasts had no more than about 0.002 CFU/g. In the dices, with the baseline calibration, the maximum contamination observed over all the simulations was about 1.2 CFU/g (that is 2 CFU on a dice) and 99% of the dices were predicted to be uncontaminated by *L. monocytogenes*. After the packaging step, the maximum contamination observed over all the simulations was about 0.04 CFU/g (that is 4 CFU in a packaging unit) and 95 [90-95]% of the packaging units were predicted to be uncontaminated by *L. monocytogenes*.

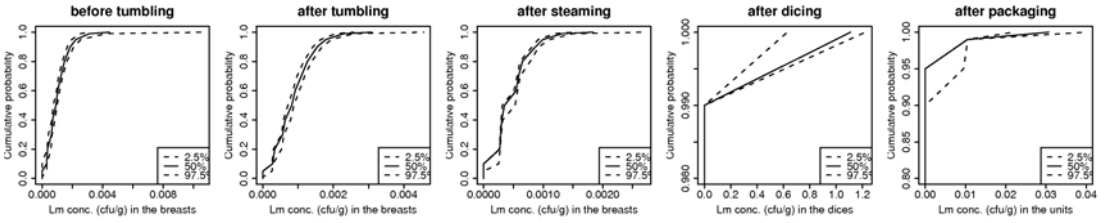

Figure 2: Cumulative distributions of the bacterial concentration (CFU/g) after the five steps already considered in Figure 1. Solid lines correspond to simulation median, long-dashed lines correspond to simulation 95% interval. The cumulative distributions must be interpreted as the repartition of items during a given step, and their variation.

The results obtained with the five scenarios alternative to the baseline calibration are summarized in Table 1. In the first three alternative scenarios, we tested changes in the process at the steaming, dicing or packaging steps. The contamination of the incoming breasts was not modified compared to the baseline calibration. Regarding the outcoming packaging units, the increase in packaging unit weight (S1) and the decrease in dice section (S2) reduced the model variability. For (S1), the 95% contamination is about 5.10^{-3} UFC/g that is 1 CFU in 200g instead of 1.10^{-2} for the baseline calibration (in the 2.5% most pessimistic simulations), that is 1 CFU in 100g. With the scenario (S2), the 95% contamination is about 1.10^{-2}, that is 1 CFU in 100g, in all the simulations, not only in the pessimistic ones. Indeed, in the scenario

(S2), much more dices were produced, and the packaging units gathered more dices than in the baseline calibration, so the contamination was homogenized within the packaging units of a batch. With the scenario (S3) the 95% contamination of the packaging units is reduced to 0. So an increase of 5°C in the steaming temperature seems to drastically inactivate the bacteria.
The last two tested scenarios concerned the breast contamination at arrival. Multiplying by 10 this initial contamination (S4) lead to a 95% concentration about 2.10^{-2}, that is 2 CFU in 100g. However the median contamination of the packaging units remained null, in contrast to the scenario (S5) (one hundred times higher initial contamination), where almost all the packaging units were contaminated.

Table 1: *L. monocytogenes* 5%, median and 95% concentration (median [95% extreme] simulation) in 10^{-3} CFU/g on the breasts at arrival (process chain inputs) and in the packaging units (process chain outputs), according to the alternative scenario.

	Incoming breasts			Outcoming packaging units		
Scenario	5%	median	95%	5%	median	95%
Baseline	0 [0-0.29]	0.91 [0.73-0.99]	2.2 [1.9-2.6]	0 [0-0]	0 [0-0]	0 [0-9.5]
S1						5.0 [5.0-5.1]
S2						9.7 [9.6-9.8]
S3						0 [0-0]
S4	5.3 [4.8-5.7]	9.1 [8.6-9.6]	17 [15-20]			20 [19-20]
S5	62 [59-65]	90 [86-94]	170 [150-190]	10 [10-11]	42 [41-47]	90 [85-95]

Discussion

In this study, we simplified the step combination, since we followed tumbling batches without considering overlapping between them during the process. In reality, the step chain is complicated by practical constraints, both in terms of time and in terms of batch overlapping. In order to refine the step combination, it would be necessary to develop a process chain manager model, taking into account open stocks and carrying capacities at every step.
To date, we neglected bacterial growth, arguing that growth was not possible during the process, given the environmental physico-chemical conditions and durations of the successive steps. However, if we want to simulate a storage step accidentally long at an accidentally high temperature, bacterial growth could occur. Also if we want to extend the model to the post-process storage until consumption, by considering further steps such as refrigerated transport and home storage, a growth model would be necessary, and potential competition between *L. monocytogenes* and the lactic acid bacteria shoud be considered (see Cornu et al., same conference). As this model enables us to compare various scenarios, it could be used to update the optimisation of the monitoring (see Commeau et al., same conference) and for re-engineering.

Conclusions

In this work, we propose a model for the diced bacon process chain, from incoming breasts to outcoming packaging units, in order to evaluate the impact of each step on the contamination.

Acknowledgements

This work was supported by a grant from the Agence National de la Recherche (ANR) (France), as a part of the Quant'HACCP project.

References

Nauta M.J. (2001) A Modular Process Risk Model Structure for quantitative microbiological risk assessment and its application in an exposure assessment of *Bacillus Cereus* in a REPFED. RIVM Report, Bilthoven, 100 pp.

Cornu M., Billoir E., Bergis H., Beaufort A. and Zuliani V. Modeling microbial competition in foods. Application to the behaviour of *Listeria monocytogenes* and lactic acid flora in diced bacon. *Submitted to the same conference.*

Commeau N., Parent E., Billoir E., Zuliani V. and Cornu M. Modelling contamination to build a sampling plan : application to French diced bacon industry and *Listeria monocytogenes. Submitted to the same conference.*