Stochastic, compartmental, and dynamic modeling of cross-contamination during mechanical smearing of cheeses

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Stochastic, Compartmental, and Dynamic Modeling of Cross-Contamination During Mechanical Smearing of Cheeses

Fanny Aziza,[1,2,*] Eric Mettler,[2] Jean-Jacques Daudin,[3] and Moez Sanaa[1]

Cheese smearing is a complex process and the potential for cross-contamination with pathogenic or undesirable microorganisms is critical. During ripening, cheeses are salted and washed with brine to develop flavor and remove molds that could develop on the surfaces. Considering the potential for cross-contamination of this process in quantitative risk assessments could contribute to a better understanding of this phenomenon and, eventually, improve its control. The purpose of this article is to model the cross-contamination of smear-ripened cheeses due to the smearing operation under industrial conditions. A compartmental, dynamic, and stochastic model is proposed for mechanical brush smearing. This model has been developed to describe the exchange of microorganisms between compartments. Based on the analytical solution of the model equations and on experimental data collected with an industrial smearing machine, we assessed the values of the transfer parameters of the model. Monte Carlo simulations, using the distributions of transfer parameters, provide the final number of contaminated products in a batch and their final level of contamination for a given scenario taking into account the initial number of contaminated cheeses of the batch and their contaminant load. Based on analytical results, the model provides indicators for smearing efficiency and propensity of the process for cross-contamination. Unlike traditional approaches in mechanistic models, our approach captures the variability and uncertainty inherent in the process and the experimental data. More generally, this model could represent a generic base to use in modeling similar processes prone to cross-contamination.

KEY WORDS: Cheese smearing; cross-contamination; modeling; Monte Carlo simulations

1. INTRODUCTION

At regular intervals during ripening, the surface of smear-ripened cheeses are salted and washed with brine containing fermenting microorganisms and sometimes alcohol (smearing solution). This is specifically to enhance the organoleptic development of the product[(1)] and to remove the undesirable molds that can develop on the surface of the cheeses. The smearing operation is carried out either manually or mechanically and causes indirect contact between cheeses via hands or a brush-smearing machine. The potential for cross-contamination, defined in this article as the transfer of microorganisms from one product to another caused by direct or indirect contact, is therefore high when undesirable microorganisms are present in one or more cheeses. In the hazard analysis and critical control point (HACCP) context, this processing step has been

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qualitatively identified as a step where products can be contaminated.

Several outbreaks of listeriosis have been attributed to the consumption of soft cheeses contaminated by *Listeria monocytogenes*.[2] Smear-ripened cheeses have been implicated in at least four such cases.[3–6] Moreover, Rudolf and Scherer observed that 15.8% of European smear-ripened cheese samples of various types contained microorganisms of the genus *Listeria*.[7] In this situation, quantitative risk assessment (QRA) models of human listeriosis linked to consumption of soft cheese made from raw milk were conducted.[8,9] The models developed assess the prevalence and concentration of *Listeria monocytogenes* in cheeses throughout the various steps of production from farm to table. Only contamination arising from the raw milk was taken into account. Indeed, it was assumed that for the type of cheese studied, white-rind soft cheeses made with raw milk (notably Camembert and Brie de Meaux), the impact of cross-contamination was negligible when good manufacturing procedures and HACCP were observed since contamination of these cheeses is almost exclusively localized in their core. However, in contrast with raw milk products, the safety of soft cheeses made with pasteurized milk is more concerned with cross-contamination since contamination of the product surface can occur during both processing and maturation. Operations such as deliberate smearing of cheese surfaces increase the risk of undesirable microorganisms being present on the product surfaces. Taking such operations into account would significantly alter the results of any QRA of pathogens in such cheeses made with raw or pasteurized milk. Finally, using QRA to quantify the potential for cross-contamination of this process, offers a means of overcoming limitations of qualitative HACCP identification.[10]

Among the six basic processes defined by Nauta in the modular process risk model,[11] cross-contamination is one of the most complex, and relatively few quantitative studies have addressed it. This article presents a compartmental modeling approach of the mechanical brush-smearing operation. The model assesses the prevalence and concentration of undesirable microorganisms on smear-ripened cheeses after the smearing step for a given scenario of the initial contamination of a batch. The transfer parameters were derived from experiments conducted with an industrial brush-smearing machine. Monte Carlo simulations were used to quantify the impact of brushing on cross-contamination. Our approach is designed to capture variability and uncertainty arising, respectively, from the process itself and from transfer parameter distributions.

2. MATERIALS AND METHODS

The model describes the transfer of microorganisms between cheeses, the machine, and the environment of the machine. It is a system of discrete deterministic difference equations. Parameters of the equations are based on physical phenomenon, making the model mechanistic.[12,13] To obtain values on parameters of the model, we conducted experiments under industrial conditions. As experimental data collected were dependant on measurement error and repetitions, we revised these data using Monte Carlo simulations and obtained empirical distributions for transfer parameters. Equations of the model and transfer parameter distributions could then be applied to any potential undesirable microorganism present on the surface of the cheeses. All calculations were performed with MATLAB® Version 6.5, Release 13.

2.1. Fundamental Concept of the Model

The fundamental concept of the model is the description of biomass transfer between cheeses, brush-smearing machine, and environment. Biomass is made up of organic matter and microorganisms, notably industrial culture strains added during manufacture. In the smearing machine, some biomass is transferred from the surface of the cheese to the machine and a fraction of it can be transferred to the following cheeses and to the environment.

Some pathogenic microorganisms (e.g., *Listeria monocytogenes*) or spoilage microorganisms (e.g., molds) may be sporadically present in the biomass on the surface of the product. Assuming that low levels of these undesirable microorganisms are homogeneously present throughout the biomass, their transfer through brushing can then be estimated by the transfer of biomass. Hence, if the proportion of biomass transferred from one compartment to another is equal to p ($p \in [0, 1]$), then the proportion of undesirable microorganisms transferred is also equal to p. The aim of the experiments therefore was to assess parameters of the model with a cheese for which the main industrial culture strain constituting the biomass could be quantified. These parameters can then be applied to any potential population of undesirable microorganisms present in the biomass.

2.2. Experimental Data Collection

In this study, we used smear-ripened cheeses made from pasteurized milk from the north of France. The cheese dimensions are $8 \times 8 \times 4$ cm and its weight is 200 g. Manufacture and ripening of this cheese under regular industrial conditions consist of the following steps: the industrial culture strain *Brevibacterium linens* BL1, a coryneform bacteria that contributes to the yellow-red stain of the rind, is inoculated into the milk ($10^{4.7}$ colony forming units (CFU)/mL). This bacteria is the main component of the cheese surface. Next, at regular stages during ripening (5, 9, 14, and 17 days), cheeses are transported on a conveyor belt and are brushed mechanically with a smearing solution in the brush-smearing machine. During the first two smearing operations (5 and 9 days), BL1 is also added to the smearing solution ($10^{7.6}$ CFU/mL).

For the experiments, two groups of cheeses were manufactured. Manufacture and ripening of the experimental cheeses were carried out under regular industrial conditions, as described above. Cheeses from the first group (called "CBL1s") were manufactured with the standard industrial culture strain, *Brevibacterium linens* BL1, whereas cheeses from the second group (called "CBL2s") were manufactured with another strain of *Brevibacterium linens*, BL2 instead of BL1. Previous studies (unpublished data) have shown that both BL1 and BL2 strains have the same growth rate on the surface of the cheese and that BL2 colonies are easily distinguishable from BL1 colonies. As *Brevibacterium linens* bacteria is one of the main components of the biomass of the cheese surface, transfer of biomass was thus observed through this bacteria. The particular strain BL2 was used as a marker in quantifying transfer parameters.

The experiments were carried out using 17-day-old cheeses. The machine was cleaned (closed circuit) and the brushes were disinfected prior to use, using industrial techniques. Five times in a row during a processing run, one CBL2 followed by 30 CBL1s were submitted to the smearing step. The first cheese (CBL2) to pass through the clean machine was collected to measure the residual population of BL2 on its surface. For each of the five series, four CBL1s from the first six CBL1s following the CBL2 were collected and the transferred populations of BL2 were measured. Before brush smearing, three CBL2s were used to assess the initial BL2 population of CBL2s.

The microbial analysis of the cheese surface was carried out by removing the whole biomass with a spatula. This biomass was decimally diluted in a sterile stomacher bag and the resulting suspension was homogenized in a stomacher blend for 2 minutes. An appropriate dilution of the suspension was plated on Brain Heart Infusion Agar (Difco) containing 50 g of NaCl per liter supplemented with pimaricin (200 ppm), nalidixic acid (40 ppm), and furazolidone (10 ppm) to inhibit yeasts, gram-negative bacteria, and staphylococci, respectively. Plates were incubated at 20°C for 15 days in order to improve the pigment formation of the colonies. The developing population was constituted by coryneform bacteria, including BL1 and BL2. Specific counts of BL2 were determined using the specific red color of the colonies and were expressed in CFU per cheese surface.

After the treatment of the five series, the inside surface of the bottom of the machine under the conveyor belt was cleaned and an additional series of 30 CBL2s was treated. Afterward, the surfaces of the brushes and the conveyor belt were swabbed with sterile sponges. The inside surface of the bottom of the machine under the conveyor belt surface was considered as the main part of the neighboring environment of the machine and was therefore also swabbed. The sponges were then immersed in 20 mL of Quarter's Strength Ringer™ solution (AES, Combourg, France) and the resulting suspension was homogenized in a stomacher blend for 2 minutes. The suspension was analyzed as described above to enumerate BL2 population.

2.3. Deterministic and Compartmental Model for Cross-Contamination During Cheese Brush Smearing

A transfer of biomass takes place between three compartments: the *machine*, the neighboring *environment*, and the *cheese* surface (Fig. 1). It occurs because of cheese-contact surfaces or because of brine

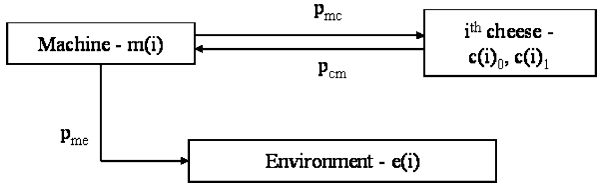

Fig. 1. Cross-contamination model. Biomass is transferred between the *machine*, the *cheese* being treated, and the neighboring *environment*. p_{kl} is the proportion of biomass transferred from compartment k to compartment l. ($k, l \in \{m, c, e\}$, standing for "machine," "cheese," and "environment," respectively.) $m(i)$ and $e(i)$ are the number of CFU on the machine and in the neighboring environment, respectively, after the cheese with rank i was treated. The cheese with rank i has two states: $c(i)_0$ and $c(i)_1$, i.e., the number of CFU before and after brush smearing, respectively.

conveying biomass through the machine. Biomass can be transferred from the *cheese* surface to the *machine* by the brushes and/or the conveyor. Such transfer can take place on cheese-contact surfaces, at points of direct contact with mechanical devices (brushes, conveyor), or through indirect contact (inner walls of the machine). The biomass can then be deposited on the following *cheese* surfaces by the *machine*. Finally, some biomass can be transferred from the *machine* to the neighboring *environment*: a part of this biomass is recovered on the inside surface at the bottom of the machine under the conveyor belt where brine flows; the other part goes onto the floor around the machine. It is assumed that the biomass does not go back into the *cheese*/*machine* subsystem from the neighboring *environment*.

The cross-contamination model is a set of three deterministic discrete difference equations, system (S) below, formulating the number of CFU of undesirable microorganisms present in each compartment after brush smearing of the cheese with rank i (the rank of the ith cheese of a batch is "i"). The population of a compartment after brush smearing of the cheese with rank i depends on: (1) the population of the compartments after brush smearing of the cheese with rank $(i-1)$ and (2) the population of the cheese i before brush smearing. Transfer parameters of the model are the proportion of biomass transferred from one compartment to another, which were assessed using the *Brevibacterium linens* population as an indicator for biomass of the cheese surface.

$$(\mathrm{S})\begin{cases} m(0) = e(0) = 0 \\ m(i) = (1 - p_{mc} - p_{me}) \times m(i-1) + p_{cm} \\ \qquad \times c(i)_0, \quad i \geq 1 \\ e(i) = e(i-1) + p_{me} \times m(i-1), \quad i \geq 1 \\ c(i)_1 = (1 - p_{cm}) \times c(i)_0 + p_{mc} \\ \qquad \times m(i-1), \quad i \geq 1 \\ 0 < p_{cm}, p_{mc}, p_{me} < 1; \quad p_{mc} + p_{me} < 1 \end{cases}$$

$m(i)$ and $e(i)$ are, respectively, the numbers of CFU on the machine and in the neighboring environment after the cheese with rank i is brushed. The cheese with rank i has two states: $c(i)_0$ and $c(i)_1$, respectively, the numbers of CFU on its surface before and after brushing. For $i \geq 1$, $c(i)_0$ are the input of the model and $c(i)_1$ are the outputs of the system (S). p_{cm} is the proportion of biomass transferred from the *cheese* to the *machine*, p_{mc} is the proportion of biomass transferred from the *machine* to the *cheese*, and p_{me} is the proportion of biomass transferred from the *machine* to the *environment*.

At the beginning, both machine and environment are clean so that $m(0) = e(0) = 0$. Constraints are: (1) parameters are the proportions of biomass transferred and must therefore be between 0 and 1, and (2) $p_{mc} + p_{me} < 1$, if not, the machine could transfer more CFU than it contains.

2.4. Model Analysis

Based on the system (S), we obtained an analytical solution (Equations (1)–(3) and (4)) for the set of parameters (p_{cm}, p_{me}, p_{mc}) for the following scenario: before smearing, only the first cheese of a batch carries some CFU on its surface, and, after smearing, the following cheeses are contaminated (cross-contaminated cheeses) because of the first cheese and, thus, can carry some CFU on their surface (if $i = 1, c(i)_0 > 0$, otherwise, $c(i)_0 = 0$ but $c(i)_1$ might be positive). This scenario fits that of the experiments performed: the BL2 population of CBL2s represents a marker of the cheese biomass; it is quantified in CFU; the first cheese of each series is manufactured with BL2, instead of BL1. Applying the analytical solution to the BL2 population thus made it possible to assess the transfer parameters of the model.

Demonstration of the following expressions[4] is detailed in Appendix A.

$$(\mathrm{S}')\begin{cases} p_{cm} = 1 - 10^{\log(c(1)_1) - \log(c(1)_0)} & (1) \\ p_{mc} = 10^{A - \log(p_{cm} \times c(1)_0) + 2B} & (2) \\ p_{me} = 1 - 10^{B} - p_{mc} & (3) \end{cases}$$

and

$$\log(c(i)_1) = A + i \times B, \quad i \geq 2. \qquad (4)$$

Equation (4) makes it possible to obtain values on parameters A and B by means of a linear regression (least square fitting) between $\log(c(i)_1)$ and i, as if i were a continuous variable and for $i \geq 2$. Equation (1) makes it possible to assess p_{cm}. Finally, Equations (2) and (3) provide values of p_{me} and p_{mc}.

2.5. Monte Carlo Simulations for Transfer Parameters Estimation

The experimental data displayed two sources of variation. The first one was uncertainty on BL2 counts,

[4] Each evocation of the logarithm (with "logarithm" or "log") in the following text corresponds to the decimal logarithm.

assumed to be normally distributed on a logarithmic scale. As measurement error was taken to be ±0.3 log CFU,[14] the mean and the standard deviation of the uncertainty distribution were, respectively, 0 and 0.15 log CFU. To take this measurement error into account, we ran Monte Carlo simulations to generate new sets of data based on experimental data corrected with a random normal additive factor (RNAF) with mean 0 and standard deviation 0.15.

The second source of variation came from the variability of the initial BL2 population of CBL2s before brush smearing. A gamma distribution, also corrected with RNAF, adequately matched these data. The same procedure was applied to the BL2 count of the first CBL2 brushed corrected with RNAF.

The parameter assessment procedure is detailed in Fig. 2. "c_exp" data stands for experimental data; "c_rev" data stands for revised data (correction with RNAF and, depending on the data, gamma adjustment). Equation (4) was adjusted on revised data for $i \geq 2$, providing values on A and B. Equation (1) was applied to revised data for $i = 1$ and made it possible to assess the transfer parameter p_{cm}. Finally, Equations (2) and (3), which require values for previously assessed p_{cm} and $c(i)_0$, made it possible to calculate p_{mc} and p_{me}. Monte Carlo simulations provided empirical distributions on transfer parameters of the model that we chose not to parameterize. Finally, Pearson's correlation coefficients between the empirical distributions of parameters were calculated.

2.6. Simulating Cross-Contamination for Specific Scenarios

Simulating cross-contamination processes requires a given scenario describing the type of initial contamination of the batch studied: the number of cheeses brushed between two cleanings (NR), the number of contaminated cheeses (N), their rank in the batch (R), and their contaminant load (C), i.e., the number of CFU of the undesirable microorganisms spread in the biomass of the surface of the contaminated cheeses.

To establish the impact of smearing on cross-contamination, we observed results for two different

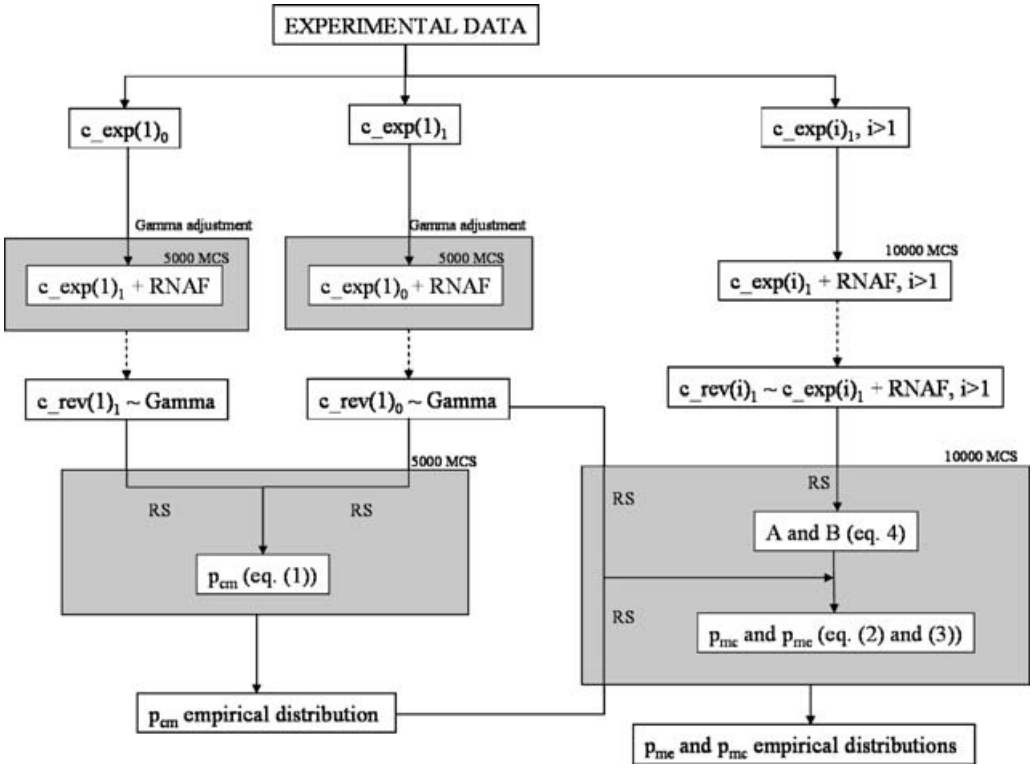

Fig. 2. Parameters estimation procedure. Experimental data ("c_exp") are corrected to take uncertainty and variability arising from the experiments into account, resulting in revised data ("c_rev"). Using Monte Carlo simulations (MCS), analytical solution of system (S) is adjusted on random sample (RS) from the distributions obtained on revised data, thus making it possible to assess transfer parameter values.

recontamination scenarios likely to occur.[13,15] The first scenario (scenario S1) is a sporadic contamination of the batch with a pathogenic microorganism: the environment contaminated the surface of one product of the batch (recontamination with water droplets, for instance). The second scenario (scenario S2) is a batch homogeneously contaminated with molds: all cheeses are contaminated with the same contaminant load on cheeses. This situation may arise because of a recontamination of the product at the beginning of processing (recontamination of coagulated milk during molding or milk contaminated because of faulty pasteurization).

For scenarios S1 and S2, we chose the contaminant load $C = 300$ CFU/cheese (2.48 log CFU/cheese). Finally, characteristics for scenarios S1 and S2 were $\{N = 1, NR = 2{,}000, C = 300, R = 10\}$ and $\{N = NR = 2{,}000, C = 300, R = [1, N]\}$, respectively. After each batch had been brushed, outputs were the new prevalence and the new allocation of CFU on the cheese surfaces.

Simulations can be implemented deterministically using system (S) (or using Equation (4) for S1) with one set of parameters (after each transfer, the number of CFU transferred must be rounded to the nearest integer). They can also be implemented stochastically using random sets of parameters from their assessed empirical distribution and applying a binomial distribution to the number of CFU transferred from one compartment to another. Indeed, the transfer of a CFU from one compartment to another can be considered as an event occurring with a probability p, p being the proportion of biomass transferred from one compartment to another. The random variable, equal to 1 if the CFU is transferred, and to 0 otherwise, follows a Bernoulli distribution with parameter p. The sum of n independent random variables following a Bernoulli distribution with parameter p follows a binomial distribution with parameters n and p. As the CFU of a compartment are independent, the number of CFU transferred follows a binomial distribution, the parameters of which are the initial number of CFU in the first compartment and the transfer parameter p. The binomial process, applied each time a transfer of CFU occurs, makes the model stochastic.

To compare results from the theoretical model and the experiments, we also simulated the scenario of the experiments conducted. Five hundred simulations of the following scenario were performed: five series of one CBL2 each followed by 30 CBL1s were treated in the machine. After these five series, the neighboring environment was cleaned (after the 35th cheese, the environment was put to 0, i.e., $e(35) = 0$), and 30 CBL2s were then treated in the machine. We compared experimental and simulated results: after the five series for the machine contamination, and after the five series and the 30 CBL2s for the environment contamination.

2.7. Analytical Results for Cross-Contamination

Using the analytical solution of the model, we derived the number of contaminated cheeses after brush smearing as a function of the transfer parameters (p_{cm}, p_{me}, p_{mc}) and the contaminant load C. This solution was calculated for a scenario S1, where the first cheese of a batch is contaminated with C CFU ($R = 1$). When the first cheese of the batch is initially contaminated with C CFU, the last cross-contaminated cheese ($i = I$) has got the lowest number of CFU on its surface. This number is greater than one, thus, $I \in \{i \geq 2 / c(i)_1 \geq 1\}$, i.e., $I \in \{i \geq 2 / \log(c(i)_1) \geq 0\}$. According to Equation (4), which provides the contaminant load of cheeses with rank $i \geq 2$, $I \in \{i \geq 2/A + iB \geq 0\}$. $f(i) = A + iB$ being a monotone decreasing function for $i \geq 2$ ($B < 0$, since $1 - p_{me} - p_{mc} < 0$), the minimum of this function is solution of $\{i \geq 2/A + iB = 0\}$. I being the rank of the last cross-contaminated cheese, it is also equal to the total number of cross-contaminated cheeses issuing from one initially contaminated with C CFU (see Appendix B for details)

$$I = 2 - \frac{\log(p_{mc} \times p_{cm} \times C)}{\log(1 - p_{mc} - p_{me})}. \tag{5}$$

For the same scenario, the ratio $J = [\max_{i \geq 1}(c(i)_1)/c(1)_0]$ stands for the maximum proportion of the contaminant load observed on contaminated cheeses compared to the initial contaminant load C. As the contaminant load of cross-contaminated cheeses decreases linearly as a function of i, the cheese with rank $i = 2$ has the highest contaminant load among cross-contaminated cheeses, i.e., $\max_{i \geq 2}(c(i)_1) = c(2)_1$. According to Equation (A.2) of Appendix A, $[c(2)_1/c(1)_0] = p_{cm} \times p_{mc}$. For $i = 1$, the proportion of the final contaminant load of the initially contaminated cheese compared to the initial is $c(1)_1/c(1)_0 = 1 - p_{cm}$, according to Equation (A.1) of Appendix A. Finally,

$$J = \max(p_{cm} \times p_{mc}, 1 - p_{cm}). \tag{6}$$

Once more for this scenario, we calculated an indicator K corresponding to the number of cheeses brushed before the number of CFU on a cheese was

reduced to 50% of the contaminant load of the first cross-contaminated cheese, i.e., the one with rank $i = 2$. This indicator was defined and used by Christensen and Rosenquist under the name B_{half}[16,17]: B_{half}, for which the actual value was unknown, was thought to influence consequences of cross-contamination of *Campylobacter* between chicken carcasses during slaughtering. They therefore tested different values to observe the impact of this factor on the risk of human campylobacteriosis. We calculated an analytical expression of K from the system (S) (see Appendix C for details):

$$K = 2 - \frac{\log(2)}{\log(1 - p_{mc} - p_{me})}. \quad (7)$$

For scenario S2, where cheeses of the batch are all contaminated with the same contaminant load C, we calculated the reduction rate L of the contaminant load of cheeses, i.e., for cheese with rank i, $L = 1 - [c(i)_1/c(i)_0]$. The expression obtained is (see Appendix D for details):

$$L = p_{cm} - p_{mc} \times \frac{1 - B_L^{i-1}}{1 - B_L}, \quad (8)$$

with $B_L = 1 - p_{mc} - p_{me}$ and $i \geq 1$.

3. RESULTS

3.1. Experimental Data

Due to microbiological technical problems, one of the series was excluded from the calculations. For the four remaining series, Table I shows the logarithm of BL2 counts on cheeses brushed. Index i is the rank of the cheese brushed. Cheeses with rank $i = 1$ were produced with BL2 strain (CBL2s). Otherwise ($i \geq 2$), cheeses were produced with BL1 strain (CBL1s). Measurement of the BL2 population on three CBL2 surfaces before brush smearing gave 8.99 log CFU, 9.22 log CFU, and 9.79 log CFU. BL2 population on the brushes and conveyor belt after the five series was 9.45 log CFU while BL2 population at the bottom of the machine under the conveyor belt was 10.54 log CFU after the five series followed by 30 CBL2s.

Table I. BL2 Population on Cheese Surfaces After Brush Smearing

	BL2 Population (log CFU)			
i	Series 1	Series 2	Series 3	Series 4
1	7.53	–	–	–
2	8.2	8.51	7.85	8.19
3	7.72	8.48	7.81	8.12
4	7.78	8.01	8.02	–
5	7.82	7.84	7.9	–
6	–	–	–	8.15
7	–	–	–	8.02

Note: These experimental data represent the logarithm of $c(i)_1$ (log CFU), for $i \geq 1$. Series 1–4 are the repetitions of the experiments.

3.2. Parameters Estimation

Fig. 3 shows cumulative gamma distributions (dotted line) adjusted on the cumulative empirical distributions of the logarithm of the BL2 population of CBL2s, corrected with RNAF (solid line), both before and after brush smearing. Parameters of the gamma distributions of BL2 counts before and after brush smearing were, respectively, {643.2, 0.014} and {2,472.5, 0.003}. Mean values of these distributions before and after brush smearing were, respectively, 9.33 log CFU ($IC_{95\%} = [8.62, 10.04]$) and 7.52 log CFU ($IC_{95\%} = [7.23, 7.84]$).

Each simulation generated four series of revised data, c_rev(i), $i \geq 2$. Fitting the linear model individually on each series provided positive slopes, whereas the analytical solution for transfer parameters required a negative slope. For instance, the linear model adjusted on the third experimental series provided a positive slope, which was considered to be due to the binomial process and the measurement error. Consequently, the linear model was fitted simultaneously on the four series. In this case, about 4% of the 10,000 simulations provided positive slopes. Results from these simulations were deleted. The adjustments of the remaining simulations were not of high quality but enabled us to approach a middling value for the slope. As an example, Fig. 4 shows the adjustment of the linear model on experimental data. Coefficients of the resulting linear regression were then used to assess transfer parameters. Nevertheless, about 30% of them provided negative p_{me}. Sets of transfer parameters resulting from these simulations were also deleted from the final empirical distributions of transfer parameters.

The empirical distributions obtained for transfer parameters (Table II) show that a high proportion of biomass is transferred from the cheese surface to the machine (mean(p_{cm}) = 0.97), whereas only a small proportion is delivered back to the cheese (mean(p_{mc}) = 0.05). As described in system (S), a nonnegligible proportion of biomass is

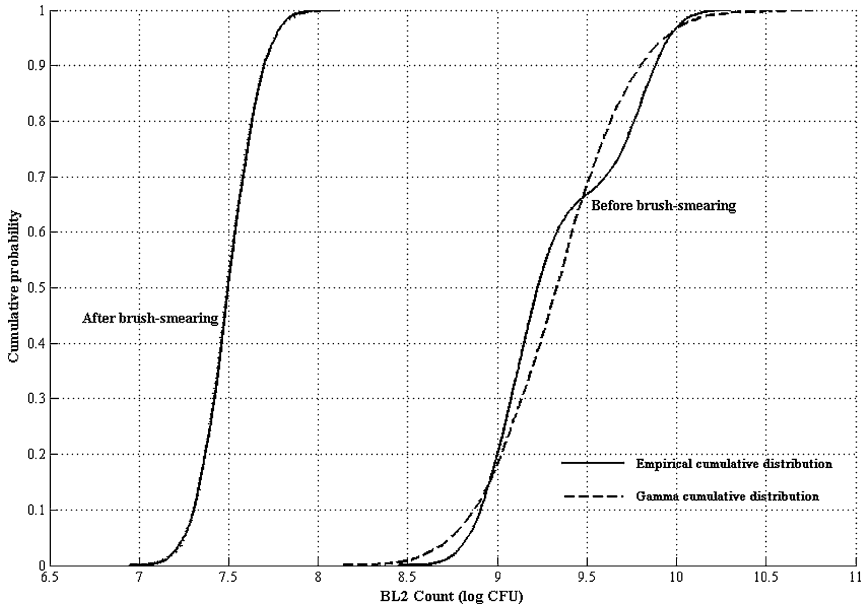

Fig. 3. Comparison of cumulative gamma distribution (dotted line) and cumulative empirical distribution (plain line) for BL2 counts before and after smearing. The gamma distribution was adjusted on the empirical distribution defined by BL2 counts corrected with RNAF before and after brush smearing.

removed principally into the neighboring environment (mean(p_{me}) = 0.07).

Correlations between parameters were not negligible. First, the correlation coefficient between the distributions of p_{cm} and c_rev$(1)_0$ was equal to −0.73. As calculations of p_{me} and p_{mc} required values on p_{cm} and c_rev$(i)_0$, we decided to take the correlation between p_{cm} and c_rev$(i)_0$ into account by sampling the values in their joint distribution. Positive correlations between empirical distributions of transfer parameters were then induced and are listed in Table III. In the same manner, sets of transfer parameters were sampled from their joint empirical distributions when simulating cross-contamination.

Regarding variance of transfer parameter distributions, lower measurement error ($< \pm 0.3$ log CFU) had little influence on the results (results not shown), suggesting that variance was induced by the intrinsic variability of the experimental data, notably BL2 population of CBL2s.

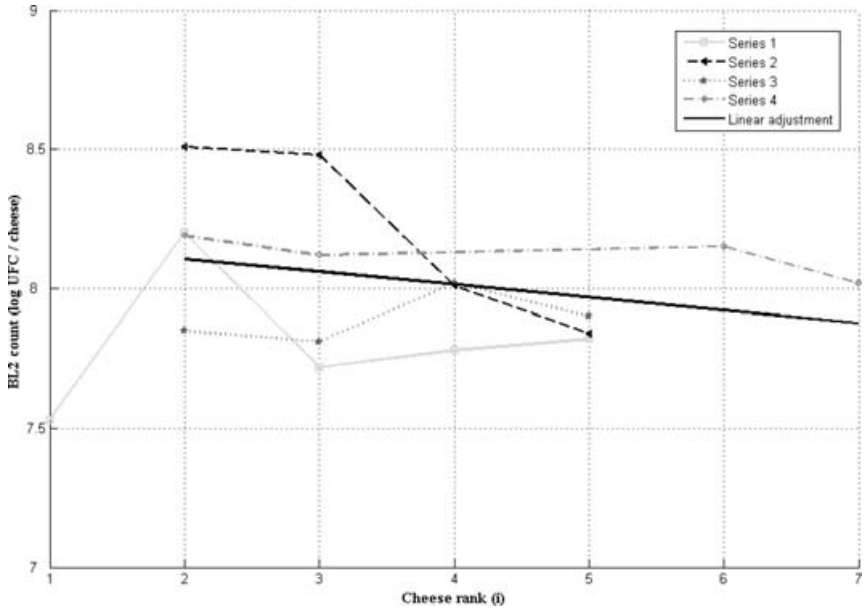

Fig. 4. Linear adjustment (least square fitting) on experimental data. The intercept on the vertical axis and the gradient are 8.2 and −0.05, respectively, parameters A and B of Equation (4). The linear adjustment is made here simultaneously for the four experimental series.

Table II. Empirical Percentiles of Transfer Parameter Distributions

Transfer Parameter	Percentiles (10,000 Simulations)				
	2.5%	25%	50%	75%	97.5%
p_{cm}	0.96	0.98	0.98	0.99	0.99
p_{me}	0.00	0.03	0.06	0.10	0.16
p_{mc}	0.01	0.02	0.04	0.06	0.12

Table III. Correlation Coefficient Between Transfer Parameter Distributions

	p_{cm}	p_{me}	p_{mc}
p_{cm}	1	0.33	−0.82
p_{me}	0.33	1	−0.36
p_{mc}	−0.82	−0.36	1

3.3. Simulating Cross-Contamination for Specific Scenarios

For scenarios S1 and S2, three sets of parameters were sampled from their joint empirical distributions and, for each random set of parameters, three simulations were performed using the binomial process. Figs. 5A–C, respectively, provide the evolution of the contaminant loads of the machine, the environment, and the cheeses simultaneously. Contaminant loads are represented on a logarithmic scale in log CFU (ordinates axis) in function of the rank of the cheese (abscissa axis). The shading of the curves are different from one set of transfer parameters to another.

Results for S1 show the linear decrease in the logarithm of the contaminant load on cross-contaminated cheeses (cheeses not initially contaminated) in function of their rank. Fluctuations during the decrease were due to the binomial process. As in the experimental data (Table I), the contaminant load of a cheese can be higher than that of the previous cheese because of both the binomial process and measurement error. The prevalence of contaminated cheeses increases highly and their contaminant load is much lower than 300 CFU.

Monte Carlo simulations using random sets of transfer parameters showed that the mean and standard deviation of the maximum proportion of contaminant load observed on cross-contaminated cheeses compared to the initial contaminant load were, respectively, 4.7% and 3.0%. Less than 2% of the initial contaminant load of the initially contaminated cheese remains on its surface (mean value). Thus, the mean and standard deviation of J, taking account of the joint distributions of parameters, were 4.7% and 3.0%, respectively. In the same manner, the mean of the indicator K, calculated using Equation (7), was 9 cheeses ($IC_{95\%} = [5, 19]$).

Results for S2 indicate that contaminant load of cheeses decreases after brush smearing. After a transitional period, contaminant loads of both the machine and the cheeses level off. As expected, the contaminant loads of cheeses are lower when cheeses belong to the transitory phase. Monte Carlo simulations, taking into account transfer parameter uncertainty in Equation (8), showed that the stable phase was reached after about the 100th cheese (mean value) and that mean and standard deviation of the reduction rate L were 55% and 25%, respectively.

The variance of the results comes from both the joint distribution of transfer parameters and the binomial process. This paragraph describes the influence of transfer parameter uncertainty and the binomial process on variance results for S1. Regarding transfer parameter uncertainty, applying Equation (5) to random sets of transfer parameters in Monte Carlo simulations showed that mean and standard deviation of the number of cross-contaminated cheeses I increased linearly in function of the logarithm of C (Table IV). The contaminant load C varied between 1 and 6 log CFU since we considered that a contaminant load above 6 log CFU was not realistic for the scenario of a product's surface recontamination. The binomial process also influences variance of the results: given a set of transfer parameters, stochastic simulations with the binomial process showed that the standard deviation of I remained constant when C increased and, no matter what the set of parameters, this standard deviation did not exceed two cheeses. Thus, for stochastic simulations with both random sets of transfer parameters and the binomial process, variance of the results was mainly due to parameter uncertainty.

To conduct the experiments and define the experimental design, we previously used a model slightly different from the one presented in this article. With this previous model, we performed simulations of the scenario of one cheese contaminated with 10 log CFU. Values of transfer parameters were sampled in a uniform distribution with parameters 0 and 1. Results of the simulations showed that a maximum of 30 cheeses were necessary to clear the brush-smearing machine of the contaminant population (results not shown). This result was used in the experimental

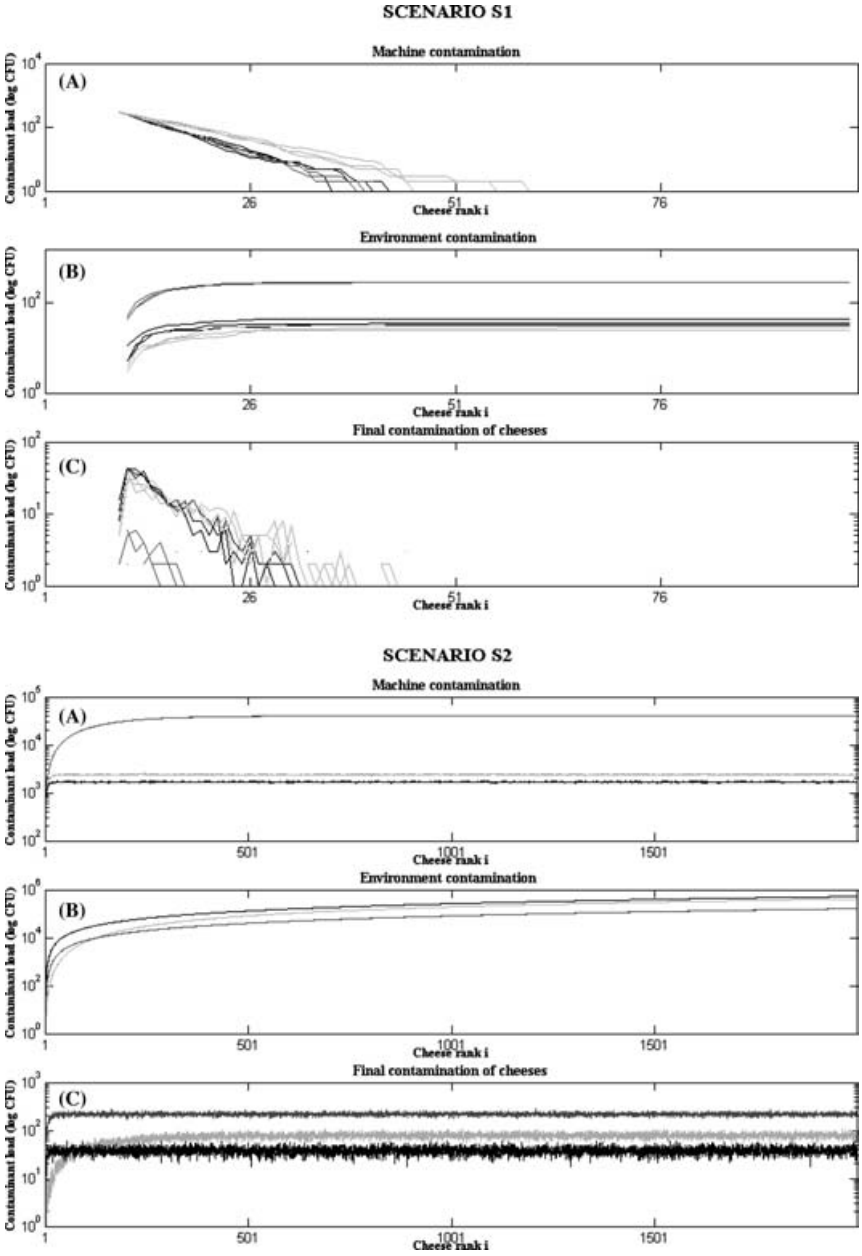

Fig. 5. Example of simulation results for scenarios S1 and S2. Three sets of transfer parameters were sampled and, for each of them, three simulations were performed using the binomial process. Each shading corresponds to a set of transfer parameters. The contaminant load of a cheese is represented on a logarithmic scale, in log CFU (ordinates axis) in function of its rank (abscissa axis). We observe the evolution of the contaminant load of the machine (A), the environment (B), and the cheeses (C) simultaneously. Characteristics of scenarios S1 and S2 are $\{N = 1, NR = 2{,}000, C = 300, R = 10\}$ and $\{N = NR = 2{,}000, C = 300, R = [1, N]\}$, respectively.

design of the experiments: between two CBL2s, 30 CBL1s passed through the machine. However, using the current model, simulation results of cross-contamination showed that more than 30 cheeses could be necessary for the machine to be cleared of the contaminant population (Table IV). We next simulated the scenario of one CBL2 followed by 30 CBL1s to calculate the BL2 population remaining in the machine after the 30th CBL1. Transfer parameter distributions were assessed using the previously presented procedure (Fig. 2) but with the first experimental series only in order to eliminate bias. The initial BL2 population of CBL2 followed the gamma distribution assessed using BL2 counts of CBL2s, before brush smearing (Fig. 3). Ninety percent of 500 simulations provided a BL2 population on the machine lower than 8 log CFU after the 30th CBL1 had been treated in the machine. Next, when using transfer parameters assessed from the four experimental series, 70% of 500 simulations gave a residual BL2 population in the machine of less than 8.2 log CFU. This residual BL2 population in the machine represented 5% of the load

Table IV. Evolution of the Number of Cross-Contaminated Cheeses I for Scenario S1, Function of the Initial Contaminant Load C

C (log CFU)	Mean (I)	SD (I)
1	2	1
2	14	6
3	36	18
4	59	30
5	81	44
6	104	59

Note: 10,000 simulations using empirical distributions of transfer parameters were performed.

taken by the brush from a CBL2 (mean value). Therefore, as the aim of these experiments was to obtain a magnitude order on transfer parameter values, we considered the influence of this bias on the independence between experimental series negligible.

The simulations of the scenario of the experiments provided differences between experimental results (BL2 population in the machine after the five series and BL2 population in the environment after the five series followed by 30 CBL1s) and simulated results. After brush smearing of the five series, the simulated mean of the machine's BL2 population was 10.55 log CFU, whereas the experimental result was 9.45 log CFU. After the whole scenario, the simulated mean of the neighboring environment's BL2 population was 10.71 log CFU, whereas the experimental result was 10.54 log CFU. These differences are due, among other things, to the fact that only a part of the machine and environmental compartments could be analyzed: regarding the neighboring environment, the inside surface at the bottom of the machine under the conveyor belt was analyzed but not the exterior of the machine (notably the floor). Regarding the machine, direct contact surfaces were analyzed (brushes and conveyor belt) but not indirect contact surfaces (notably the inner walls of the machine). Another reason for these differences is the uncertainty of the measurement method of the BL2 population.

4. DISCUSSION

Several aspects of the model should be highlighted. Concerning model validation, confidence in simulated results depends on the propensity of the theoretical model to match reality. Even if the differences between simulated and experimental results are partially related to the fact that neither the whole machine nor the complete environment could be analyzed, the fact remains that more experimental data are necessary to properly validate this cross-contamination model.

It has to be recognized that the analytical solution for transfer parameters (Equations (1)–(3) and (4)) is based on the experimental protocol: the first cheese only is contaminated (with a known contaminant load), which makes it possible to assess first p_{cm}, and then the pair (p_{mc}, p_{me}) using results on p_{cm}. As seen from the present experiments, uncertainty engendered by transfer parameters is relevant. Such a situation is due mainly to the uncertainty of the measurement method and the intrinsic variability of the experimental data. However, these experiments facilitate the development of a plausible idea for transfer parameter values. If transfer parameter uncertainty can be reduced, variance due to the binomial process can then be taken into account, paving the way for more realistic results in QRA.[18]

The approach used here is different from the traditional approach, which focuses on individual transfer rates between various surface and/or various products for a defined microorganism.[19–21] Our approach presents an advantage because we are interested in the case of limited contamination of undesirable microorganisms spread through the biomass of the product surface (induced by a recontamination event, for instance). This hypothesis leads us to assume that the undesirable microorganisms do not modify the behavior of the biomass. Thus, to assess transfer parameters, only one microorganism constituting the biomass of the product surface is necessary (here, *Brevibacterium linens*) and these transfer parameters can then be applied to any undesirable microorganism.

According to the literature, the linear decrease of the logarithm of the contaminant load on cross-contaminated products (for scenario S1) seems plausible and has already been observed. When assessing microbial populations on hands, Veulemans *et al*. observed that the logarithm of the contaminant load of the adhesive tape in contact with hands decreased linearly in function of the tape rank.[22] Midelet and Carpentier also applied this model to assess the attachment strength of microorganisms on some surfaces, including *Listeria monocytogenes*.[23] Vorst *et al*. have recently shown data that reinforced the idea of a linear decrease for scenario S1:[24] the experiments performed quantify the transfer rate of *Listeria monocytogenes* from inoculated products to uninoculated products via a slicing machine. In similar experiments,

Pérez *et al*. observe the transfer rate of *Staphylococcus aureus* from the machine to uninoculated products.[25] For both studies, a linear decrease of the logarithm of the contaminant load of cross-contaminated product in function of the slice rank is observed. Transfer rates obtained for *Listeria monocytogenes* are different from one inoculum to another. However, pure inoculums of *Listeria monocytogenes* were used, whereas the central concept of our model is that contaminant cells are diluted in the biomass of the cheese surface, so that transfer parameters do not depend on the initial contaminant load of the contaminated product. Moreover, adhesion of cells to the surface may influence results on transfer rates, which is not the case here.[26]

Christensen *et al*., when developing risk assessment on *Campylobacter spp.* in chicken products,[16,17] modeled cross-contamination between carcasses by means of linear decrease of the logarithm contaminant load on carcasses for scenario S1. However, they did not give any analysis showing that the linear regression was the proper solution. The slope of this decrease was determined by the indicator B_{half}, defined by the number of carcasses that need to be slaughtered before the number of *Campylobacter* cells on a bird was reduced to 50% of the contaminant load of the first cross-contaminated carcass. As B_{half} was currently unknown, five different values were used, from 300 to 6,000. The indicator K we used, which had the same definition as B_{half}, was analytically calculated (Equation (7)) from the system (S). Its mathematical expression did not depend on the initial contaminant load of the initially contaminated cheese but rather on only the slope of the linear regression, i.e., on p_{me} and p_{mc}. We believe that this indicator, a cross-contamination *strength*, represents an efficient summary of the cross-contamination propensity of the process and can be deduced mathematically from transfer parameters of the system (S).

Concerning the impact of brush smearing on cheese contamination, results for scenario S2 show that the initial contaminant load of a homogeneous contaminated batch will be reduced by more than a half. That result proves the efficiency of brush smearing since one aim of this processing step is to extract potential undesirable microorganisms that could arise from the environment. For scenario S1, where one cheese was contaminated, less than 2,000 cheeses were contaminated, no matter what the initial contaminant load (Table IV). Thus, in the case of the plant where experiments were conducted and where hygienic measures of the machine are planned about every 2,000 cheeses, the model indicates that this frequency of cleaning and disinfection is not sufficient to stop a cross-contamination event inside a batch. Results for scenario S1 also indicate that the contaminant load of the initially contaminated cheese is highly spread on the cross-contaminated cheeses with small contaminant loads: the contaminant load of the first cheese cross-contaminated represents less than 5% of the initial contaminant load. Thus, microbial analysis of products during the process should be performed once the detection level of the test used to search out an undesirable microorganism is known: for a test able to detect any level of population, products should be analyzed after brush smearing because of the high number of contaminated cheeses. At the same time, the model suggests that there may be a threshold combining number of contaminated cheeses and initial contaminant load above which products will be detectable before brush smearing but not after. This threshold depends on the detection level of the test for the microorganism concerned. This model could thus be used as an objective tool to improve sampling in a plant.

This model will be integrated in the future as a module in the microbial quantitative assessment of listeriosis linked to the consumption of soft cheeses made from pasteurized milk taking the whole process into account. Inputs of this model are the rank of the contaminated cheeses of a batch and their level of contamination, which come from the previous step of the process. Integration of this step will considerably improve results in listeriosis risk detection since physical and chemical parameters of cheeses during ripening, such as temperature and pH, affect the growth of *Listeria monocytogenes*[9,27] even if contaminant loads of cross-contaminated cheeses are low. However, the question of the outbreak of a CFU during brush smearing and after a growth period remains to be addressed, as we do not yet know the mechanical effect of brushing on it. Assumptions lead to two extremes: (1) a CFU is totally broken up, forming as much new CFU as the number of microorganisms it contained before brushing; or (2) a CFU is not broken up and the whole CFU is transferred. No literature is available on the splitting of colonies. Experiments should therefore be performed to gain understanding of this phenomenon and add a splitting factor to the model. Such a factor would be applied to any CFU coming from the surface of the cheese and entering the machine. As the final number of contaminated cheeses depends on the initial contaminant load of initially contaminated cheeses, splitting CFUs entering the machine would have consequences on the results.

More generally, this model may represent a generic model for cross-contamination. First, the concept of mechanistically modeling the biomass transfer coming from the product makes it possible to apply the cross-contamination model to any undesirable microorganism. Second, it is based on logical and simple ideas, compartments and transfer rates between them, which are easily recognizable for many processes prone to cross-contamination (like handling or slicing). Using an adapted marker, the same experiments can be organized. The cross-contamination *strength*, defined above and for which analytical expression is available with Equation (7), could be used to compare industrial processes prone to cross-contamination and fitting our model.

Finally, the model also highlights the correlation between contamination of the product by the environment and cross-contamination.[15] Indeed, the model shows how the environment and the machine become contaminated during the process. Sampling results from industrial plants often present a persistence of strains in the environment and/or on machines.[28,29] This persistence can be explained by the difficulty of completely eradicating undesirable microorganisms from the machine and the environment.[30] Improvements to this model could thus help to gain a better understanding of the dynamic of a plant contamination.

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APPENDIX A

The system (S) describes the number of CFU in a compartment after the cheese with rank i has been brushed.

$$(\mathrm{S})\begin{cases} m(0) = e(0) = 0 \\ m(i) = (1 - p_{mc} - p_{me}) \times m(i-1) + p_{cm} \\ \qquad \times c(i)_0, \quad i \geq 1 \\ e(i) = e(i-1) + p_{me} \times m(i-1), \quad i \geq 1 \\ c(i)_1 = (1 - p_{cm}) \times c(i)_0 + p_{mc} \\ \qquad \times m(i-1), \quad i \geq 1 \\ 0 < p_{cm}, p_{mc}, p_{me} < 1; \quad p_{mc} + p_{me} < 1 \end{cases}$$

In the case $i = 1$, as $m(0) = 0$,

$$c(1)_1 = (1 - p_{cm}) \times c(1)_0. \tag{A.1}$$

Hence,

$$\mathrm{p_{cm}} \quad 1 \quad \frac{\mathrm{c(1)_1}}{\mathrm{c(1)_0}}$$

$$\mathrm{p_{cm}} \quad 1 \quad 10^{\log(\mathrm{c(1)_1})\log(\mathrm{c(1)_0})} \quad (1).$$

Moreover, $\mathrm{m(1)} \quad \mathrm{p_{cm}} \quad \mathrm{c(1)_0}$.

In the case $i = 2$, as $c(2)_0 = 0$,

$$c(2)_1 = p_{mc} \times m(1) \Leftrightarrow$$

$$c(2)_1 = p_{mc} \times p_{cm} \times c(1)_0 \tag{A.2}$$

and

$$m(2) = (1 - p_{mc} - p_{me}) \times m(1) \Leftrightarrow$$

$$m(2) = (1 - p_{mc} - p_{me}) \times p_{cm} \times c(1)_0.$$

In the case $i \geq 3$, as $c(i)_0 = 0$, $m(i) = (1 - p_{mc} - p_{me}) \times m(i-1)$ and $c(i)_1 = p_{mc} \times m(i-1)$.

Hence,

$$c(i)_1 = p_{mc} \times (1 - p_{mc} - p_{me}) \times m(i-2).$$

This recurrence relation easily gives $c(i)_1 = p_{mc} \times (1 - p_{mc} - p_{me})^{i-2} \times m(1)$.

As $m(1) = \mathrm{p}_{cm} \times c(1)_0$,

$$c(i)_1 = (1 - p_{mc} - p_{me})^{i-2} \times p_{mc} \times p_{cm} \times c(1)_0. \tag{A.3}$$

When applying $i = 2$ to Equation (A.3), $c(2)_1 = p_{mc} \times p_{cm} \times c(1)_0$, which is the same result as Equation (A.2). Thus, Equation (A.3) works for $i \geq 2$.

Switching over to the decimal logarithmic scale led to a linear relationship between $\log(c(i)_1)$ and i, $i \geq 2$: $\log(c(i)_1) = A + i \times B, \quad i \geq 2$ (4).

A and B from Equation (4) are defined by the following system:

$$(\mathrm{S}')\begin{cases} A = \log(p_{mc} \times p_{cm} \times c(1)_0) - 2 \\ \qquad \times \log(1 - p_{me} - p_{mc}) \\ B = \log(1 - p_{me} - p_{mc}). \end{cases}$$

Finally, solving system (S′) results in the following equations

$$\begin{cases} p_{mc} = 10^{A - \log(p_{cm} \times c(1)_0) + 2B} & (2) \\ p_{me} = 1 - 10^{B} - p_{mc}. & (3) \end{cases}$$

Equation (4) makes it possible to obtain values on parameters A and B by means of a linear regression (least square fitting) between $\log(c(i)_1)$ and i, as if i were a continuous variable and for $i \geq 2$. Equation (1)

makes it possible to assess p_{cm}. Finally, Equations (2) and (3) provide values of p_{me} and p_{mc}.

APPENDIX B

Let I be the number of cross-contaminated cheeses from one initially contaminated with C CFU. $I \in \{i \geq 2 / A + iB = 0\}$ since I is the last contaminated cheese and, for $i \geq 2$, $\log(c(i)_1) = A + iB$. A and B are defined by the system (S′) (see Appendix A). Deriving $A + IB = 0$ as if I were continuous gives:

$$A + I \times B = 0 \Leftrightarrow I = \frac{-A}{B}$$

$$\Leftrightarrow I = \frac{-\log(p_{mc} \times p_{cm} \times C) + 2 \times \log(1 - p_{mc} - p_{me})}{\log(1 - p_{mc} - p_{me})}$$

$$\Leftrightarrow I = 2 - \frac{\log(p_{mc} \times p_{cm} \times C)}{\log(1 - p_{mc} - p_{me})}. \quad (5)$$

APPENDIX C

For scenario S1, indicator K corresponds to the number of cheeses brushed before the number of CFU on a cheese is reduced to 50% of the contaminant load of the first cross-contaminated cheese ($i = 2$).

Thus,

$$K = \left\{ i / c(i)_1 = \frac{c(2)_1}{2} \right\},$$

i.e.,

$$K = \{ i / \log(c(i)_1) = \log(c(2)_1) - \log(2) \}.$$

As $\log(c(i)_1) = A + iB$, for $i \geq 2$ and $c(2)_1 = p_{mc} \times p_{cm} \times c(1)_0$ (see Appendix A), we come to

$$K = \frac{\log(p_{mc} \times p_{cm} \times c(1)_0) - \log(2) - A}{B}.$$

According to the system (S′) of Appendix A,

$$K = \frac{\log(p_{mc} \times p_{cm} \times c(1)_0) - \log(2) - \log(p_{mc} \times p_{cm} \times c(1)_0) + 2 \times \log(1 - p_{me} \times p_{mc})}{\log(1 - p_{me} \times p_{mc})}.$$

Finally,

$$K = 2 - \frac{\log(2)}{\log(1 - p_{me} - p_{mc})}. \quad (7)$$

APPENDIX D

Let us calculate $c(i)_1 / c(i)_0$ for scenario S2, where all cheeses are contaminated with the contaminant load C, i.e., $c(i)_0 = C, \forall i \geq 1$. The reduction rate L is $1 - [c(i)_1 / C]$.

According to the system (S),

$$\begin{cases} c(i)_1 = (1 - p_{cm}) \times c(i)_0 \\ \qquad + p_{mc} \times m(i-1) \\ m(i) = (1 - p_{cm} - p_{me}) \\ \qquad \times m(i-1) + p_{cm} \times c(i)_0, \end{cases} \quad \text{for } i \geq 1.$$

In the case where all cheeses are contaminated with the same load C, the recurrence equation for $m(n)$, $n \geq 2$ easily gives:

$$m(n) = B_L^{n-1} \times m(1) + C \times p_{cm} \times \sum_{k=0}^{n-2} B_L^k,$$

with $B_L = 1 - p_{mc} - p_{me}$. As

$$m(1) = p_{cm} \times C \quad \text{and} \quad \sum_{k=0}^{j} B_L^k = \frac{1 - B_L^{j+1}}{1 - B_L},$$

$$m(n) = C \times p_{cm} \times \frac{1 - B_L^n}{1 - B_L}, \quad n \geq 0.$$

If $n = i - 1$, we obtain

$$c(i)_1 = (1 - p_{cm}) \times C + p_{mc} \times \frac{1 - B_L^{i-1}}{1 - B_L}, \quad i \geq 1.$$

Thus,

$$\frac{c(i)_1}{C} = 1 - p_{cm} + p_{mc} \times \frac{1 - B_L^{i-1}}{1 - B_L}, \quad i \geq 1.$$

And finally,

$$L = p_{cm} - p_{mc} \times \frac{1 - B_L^{i-1}}{1 - B_L}, \quad i \geq 1. \quad (8)$$

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