



Preliminary Quantitative Microbial Risk Assessment for Staphylococcus enterotoxins in fresh Minas cheese, a popular food in Brazil



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ABSTRACT

The main objective of this work was to assess the risk associated with staphylococcal enterotoxins (SE) intoxication after the consumption of fresh Minas cheese by the Brazilian population. Coagulase-positive staphylococci data from 350 samples were obtained from monitoring programs, and were used as a proxy for *S. aureus* contamination, considering that 73% of the strains were toxigenic. The Combined Database for Predictive Microbiology (ComBase) and the Pathogen Modeling Program (PMP) models were used to predict *S. aureus* growth rate and lag-phase in fresh Minas cheese at different pH, salt concentration and storage temperature in a household refrigerator, up to 7 days before consumption. Change in storage temperature had the largest impact on the growth rate and lag-phase obtained from both models. Cumulative probability of SE intake events equal to or higher than the toxigenic dose of 100 ng were calculated using Monte Carlo simulations performed by the @Risk software. The toxic dose was exceeded at the 99.95th percentile of exposure in the ComBase model (upper bound) for the adult population, the lowest percentile identified in the study. The *S. aureus* initial concentration was the parameter that most impacted the output obtained by @risk, indicating the importance of good manufacturing practices for fresh Minas cheese production, and proper storage conditions at the point of sale. This preliminary assessment indicated that the risk of staphylococcal intoxications from the consumption of fresh Minas cheese by the Brazilian population is probably low. The study identified many data gaps that needs to be addressed to improve the risk assessment.

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1. Introduction

Staphylococcus aureus is the most prevalent and economically significant pathogen causing intramammary infections in dairy ruminants (Nader Filho, Ferreira, Amaral, Rossi, & Oliveira, 2007; Peles et al., 2007; Wang et al., 2009). It can contaminate milk either by direct excretion from udders with staphylococcal mastitis or during handling and processing of raw milk (André et al., 2008; Wang et al., 2009). *S. aureus* is a facultative anaerobe Gram-positive, catalase and coagulase positive coccus, which can grow in a wide range of pH (4.5–9.3) and temperature (7–47.8 °C), and at water activity (a_w) as low as 0.83 (FDA, 2012). *S. aureus* strains are also highly tolerant to salts and sugar (FDA, 2012).

S. aureus produces a wide variety of toxins, including the classic staphylococcal enterotoxins (SE) with demonstrated emetic activity (SEA to SEE, SEG to SEI, SER to SET) (Argudim, Mendoza, & Rodicio, 2010; Le Loir, Baron, & Gautier, 2003). More recently, new proteins (SE-like toxin, SEIK to SEIQ) with similar amino acid sequences were demonstrated to also have emetic activities in a primate model (Omoe et al., 2013). Ono et al. (2015) identified a novel staphylococcal toxin (SEIY), which exhibited emetic activity in house musk shrews. SE are single-chain proteins with molecular weights of 24,000 to 29,000, resistant to proteolytic enzymes, which allows them to transit intact through the digestive tract, and are resistant to temperatures that would destroy the bacilli (FDA, 2012; Le Loir et al., 2003). Temperature control below 10 °C is required to inhibit SE production (Tutsuura & Murata, 2013). Other factors that affect SE production include the *S. aureus* strain, storage conditions, and type of milk (Janštová, Necidová, Janštová, & Vorlová, 2012).

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The intoxication dose of SE is less than 1000 ng, a level that is reached when *S. aureus* populations exceeds 100,000 organisms/g in food, indicative of unsanitary conditions. In highly sensitive people, ingestion of 100–200 ng of enterotoxin can cause symptoms of staphylococcal food poisoning (FDA, 2012). The symptoms include nausea, vomiting, abdominal cramps and diarrhea (Carmo et al., 2004; FDA, 2012). Although severe dehydration may occur, the illness is usually self-limiting, and recovery occurs within 24–48 h with proper supportive care (FDA, 2012; Kérouanton et al., 2007; Le Loir et al., 2003). Scallan et al. (2011) estimated that 241,188 illnesses due to *S. aureus* occur each year in the United States occurs, with 1,064 hospitalizations, and six deaths annually. In Brazil, 10,666 foodborne outbreaks were notified to the Ministry of Health from 2000 to 2014, mainly from food consumed in the household (MS, 2015). About 42% of the outbreaks had the agent identified, of which 18.5% involved *S. aureus*; milk and milk products were involved in 7% of the outbreaks with the food identified. In the investigation of two outbreaks that occurred in the state of Minas Gerais, Brazil, *S. aureus* strains showing to be producers of SEA, SEB and SEC were isolated from Minas cheese and raw milk samples (Carmo et al., 2002).

Various studies in Brazil have shown that Minas cheese presented the highest prevalence of coagulase-positive *Staphylococci* among dairy products (Carvalho, Viotto, & Kuaye, 2007; Ferreira et al., 2011; Moraes, Viçosa, Yamazi, Ortolani, & Nero, 2009; Rodrigues et al., 2011). Arcuri et al. (2010) showed that over 70% of the *S. aureus* strains isolated from fresh Minas cheese were enterotoxigenic. Typically Brazilian, Minas cheese is the most consumed cheese in the country (about 30 g/person/day; IBGE, 2011). The fresh type (*Minas frescal*) has high humidity (up to 45.9% water content), a pH between 5 and 6 (Rocha, Buriti, & Saad, 2006), and a maximum shelf life of 9 days under refrigeration (Sangaletti et al., 2009). A previous study highlighted the inadequate hygienic-sanitary conditions of the Minas cheese available for consumption in Brazil and the need to further investigate the potential risk of consumers (Nunes, Mota, & Caldas, 2013).

Quantitative Microbial Risk Assessment (QMRA) framework is a useful tool to evaluate the risk of consuming contaminated food and prevent foodborne diseases. Predictive models for microbial growth and survival under particular environmental conditions have been used for risk assessment of food-borne microorganisms (Ding et al., 2016; Fujikawa and Morozumi, 2006; Heidinger, Winter, & Cullor, 2009; Kim, Griffiths, Fazil, & Lammerding, 2009; Rho & Schaffner, 2007; Schelin et al., 2011). The extent of microbial growth is a function of the time the population is exposed to combinations of intrinsic food properties (e.g., salt concentration and acidity), and extrinsic storage conditions (e.g., temperature, relative humidity, and gaseous atmosphere) (McMeekin et al., 1997). Predictive models such as ComBase and PMP have been used by other authors to estimate the growth rate and lag-phase for QMRA studies of *S. aureus* (Heidinger et al., 2009; Lindqvist et al., 2002; Yoon et al., 2011).

The main objective of this work was to estimate the risk associated with SE exposure from the consumption of fresh Minas cheese in Brazil purchased at retail stores. Microbiological data were obtained from monitoring programs around the country and the ComBase and PMP growth models were used to simulate the contamination levels at the time of consumption.

2. Materials and methods

The QMRA process includes the hazard identification, hazard characterization (dose-response), exposure assessment and risk characterization steps. In this study the hazard was identified as the staphylococcal enterotoxins (SE), for which 100 ng was considered

the dose to cause symptoms of staphylococcal food poisoning (FDA, 2012; hazard characterization). Exposure assessment and risk characterization were conducted using the data and the models explained in the next sections.

2.1. Microbiological data on fresh Minas cheese at the time of purchase

Brazilian food legislation includes analysis of coagulase-positive staphylococci (CPS; maximum of 10^3 CFU/g for fresh Minas cheese), which is conducted under state sanitary surveillance programs. *S. aureus* and staphylococcal enterotoxin investigations are only performed in food samples suspected to be involved in foodborne outbreaks.

In this work, CPS data on fresh Minas cheese were obtained from the National Sanitary Surveillance Agency (ANVISA), which was a compilation of data from nine state laboratories on samples analyzed from 2010 to 2012. Additionally, data were obtained directly from the Central Laboratory of the Federal District (LACEN-DF) on samples analyzed between 2000 and 2014. The samples were analyzed using standard protocols (APHA, 2001).

The CPS data include results reported as zero or absent, as censored data (<3 , <10 or <100 CFU/g) and as finite enumeration. In this study, two levels of exposure were estimated: 1) the lower bound, where results reported as below 3, 10 or 100 CFU/g, zero, or absent, were assigned as 1 CFU/g, and 2) the upper bound, where levels reported as <3 CFU/g were assigned as 3 CFU/g, <10 CFU/g as 10 CFU/g, <100 CFU/g as 100 CFU/g, and those reported zero or absent as 1 CFU/g.

2.2. Fresh Minas cheese consumption

Consumption data for fresh Minas cheese were obtained from the 2008/2009 Brazilian Household Budget Survey (*Pesquisa de Orçamento Familiar*; IBGE, 2011) in which 34,003 individuals 10 years or older from all 26 Brazilian states and the Federal District completed a two non-consecutive day dietary reports. The data (portion, in g) also include information on fresh Minas cheese consumption in sandwiches, and the age of the consumer. Mean consumptions were 85.2, 88.8 and 72.3 g for teenagers, adults and seniors, respectively. In the exposure model used in this study, the variable (P) represents the cheese portion size. The histogram distribution was used to model the portion size for teenagers (10–19 years), adults (20–59 years) and elderly persons (60 years or older).

2.3. Household storage temperature, pH and % NaCl (w/w)

Bacterial growth was simulated from the time of purchase at the selling point to immediate consumption or after storage (t) in a domestic refrigerator, ranging from 1 to 168 h (7 days). A uniform distribution (continuous) was used to model the time of storage. Daily temperature records of a domestic refrigerator during a 16 months period ($n = 734$) were used to simulate the storage temperature (T) in the household. The values ranged from -0.9 to 17°C , with a mean of 4.3°C , median of 4.0°C , and mode (most likely value) of 7.8°C . The histogram distribution was used to model the temperature.

pH values of fresh Minas cheese were kindly provided by Prof. Susana Saad, from the University of São Paulo, Brazil, and concerned 76 samples obtained in the local São Paulo market. The pH values ranged from 4.9 to 6.5, with a mean and median of 5.8, and mode of 5.5. Salt concentrations of fresh Minas cheese, in % NaCl (w/w), were reported for 40 samples analyzed by the state laboratories and the LACEN-DF. Values ranged from 0.64 to 4.6% (mean of 1.28%, median of 1.14%, and mode of 1.46%).

2.4. Growth modules

Two models were used to predict *S. aureus* growth rate (μ , in log CFU/g/h) in fresh Minas cheese. The Combined Database for Predictive Microbiology (ComBase) is managed by the Institute of Food Research in the United Kingdom, the USDA Agricultural Research Service in the United States, and the University of Tasmania Food Safety Centre in Australia (ComBase, 2016). The Pathogen Modeling Program (PMP version 7) was developed by the U.S. Department of Agriculture (PMP, 2016).

The following parameters are included in the ComBase model for growth rate (static mode): the inoculum physiological state, the initial *S. aureus* level (C_{il}), pH, % NaCl (w/w), and the storage temperature (T), which in this study is the household storage temperature. ComBase, as well as the PMP, does not allow to model the parameters to generate distributions of growth rate, so fixed values of T (7.5, 10, 12.5, 15 and 17 °C), pH (5.0, 5.5 and 6.5) and NaCl concentration (1.1, 2.1, and 4.5% w/w) were tested to investigate the impact on the growth rate, and consequently on the lag-phase.

The physiological state is a dimensionless number between 0 and 1, expressing the physical suitability of the cells to their environment - when it is 0, growth will not occur (infinite lag-phase), when it is 1, growth will start immediately, without lag-phase. The lag-phase is the transition period during which microbial cells adjust to the environment before exponential growth starts (Swinnen, Bernaerts, Dens, Geeraerd, & Van Impe, 2004). The physiological state does not affect the growth rate μ , but affects the lag-phase time (λ , in hours) through the equation $\lambda = -\log(\text{physiological state})/\mu$ (ComBase, 2016). In this study, we assumed a constant physiological state of 0.5 to estimate the lag-phase for each growth rate provided in the ComBase model.

In the PMP model (Broth Culture, Aerobic), the growth rate μ was determined for the storage temperatures in the range of 10 °C (lowest allowed in the model) to 17 °C, and the same pH and % NaCl values tested in the ComBase model. The PMP model predicts a maximum population density (MPD) of 9.6 log CFU/g, and gives the lag-phase at each temperature and growth rate. In both ComBase and PMP models, a default initial *S. aureus* level of 3 CFU/g was inputted, but this value does not affect the growth rate.

Further, at a constant pH and NaCl concentration, the square root of μ at a given storage temperature (T) was plotted and the calculated linear regression was used to model the effect of temperature on growth rate for both models (GR_c and GR_p , respectively). The relationship between lag-phase and temperature was also expressed by a linear regression equation (LT_c and LT_p , respectively) (Heidinger et al., 2009).

2.5. Exposure to *Staphylococcal enterotoxin (SE)* from the consumption of fresh Minas cheese contaminated with *S. aureus*

To estimate the level of exposure to SE at the time of consumption, the growth rate formulas obtained by each model was applied to the initial *S. aureus* level found at the time of purchase (C_{ic}). The *S. aureus* contamination at the time of consumption (C_{con}) was different from the initial concentration (C_{ic}) if the initial prevalence (P_{sa}) was 1, indicating a positive sample, and the time of consumption was higher than the predicted lag-time (LT). In the PMP model, the contamination level at the time of consumption (C_{con}) is equal to C_{ic} if C_{ic} is equal to or higher than the maximum population density (MPD, 9.6 log CFU/g).

2.6. Risk characterization of the exposure to SE from the consumption of fresh Minas cheese

In this study, the minimum SE dose causing intoxication was

assumed to be 100 ng, a relevant dose for highly sensitive people (FDA, 2012). The risk is given by the cumulative probability of events of SE intake equal or higher than 100 ng occurring from the consumption of fresh Minas cheese, under the conditions considered in the study. The probabilities were calculated using Monte Carlo simulations performed by the @Risk software 6.2 (Palisade Corporation, USA), with fixed seed configuration, and Mersenne Twister generator, 100,000 iterations, 1% precision, and 95% confidence interval to the convergence point.

The variable *prevalence of toxigenic strains* (P_{tg}) was set at 73%, based on the work conducted by Arcuri et al. (2010). In this study, 51 of the 70 isolates from *frescal* Minas cheese samples were positive for at least one toxigenic gene, including the classical SE genes (*sea* through *see*), the more recently described SE genes (*seg* through *sell*), which encode for SE-like toxins, and the *tst-1* gene, which encodes for staphylococcal toxic shock syndrome toxin 1.

The variable *enterotoxin (SE)* (Tox), as log ng/g, is given by the equation $Tox = 0.9301 \cdot C_{con} - 6.6621$, obtained by Kim et al. (2009) using the growth data reported by Soejima et al. (2007) in milk products. This equation was applied to the models when the contamination level was ≥ 5 log CFU, minimum bacterial concentration for enterotoxin production (FDA, 2012).

Table 1 summarizes the formulas used to estimate the exposure and the risk with the variables and conditions assumed in this study, and Fig. 1 summarizes the approach.

3. Results

3.1. Prevalence and initial contamination level

A total of 350 samples of fresh Minas cheese were tested for coagulase-positive *Staphylococcus* (CPS), of which 18.57% had finite CPS results reported, and 81.43% of the samples were reported as <3, <10 or <100 CFU/g, zero, or absent. These percentages define the discrete distribution for the prevalence of *S. aureus* (P_{sa}) for the lower bound intake estimation. For the upper bound estimation (samples reported at <3, <10 and < 100 CFU/g were set at 3, 10 and 100 CFU/g, respectively), the prevalence was 87.43%. The highest reported value was 9.6 log CFU/g. Only two fresh Minas cheese samples collected during the investigation of a foodborne outbreak were analyzed for SE identification, and no toxin was detected in either sample.

The lower and upper bound distributions of CPS in the fresh Minas cheese, referred to in this study as the initial concentration of *S. aureus*, are shown in the top right of Fig. 1. The data characterize the contamination level at the time of purchase (C_{ic}). The initial contamination level (C_{il}) is the *initial concentration* considered at each iteration when the prevalence is positive ($P_{sa} = 1$).

3.2. Growth rates and lag phase

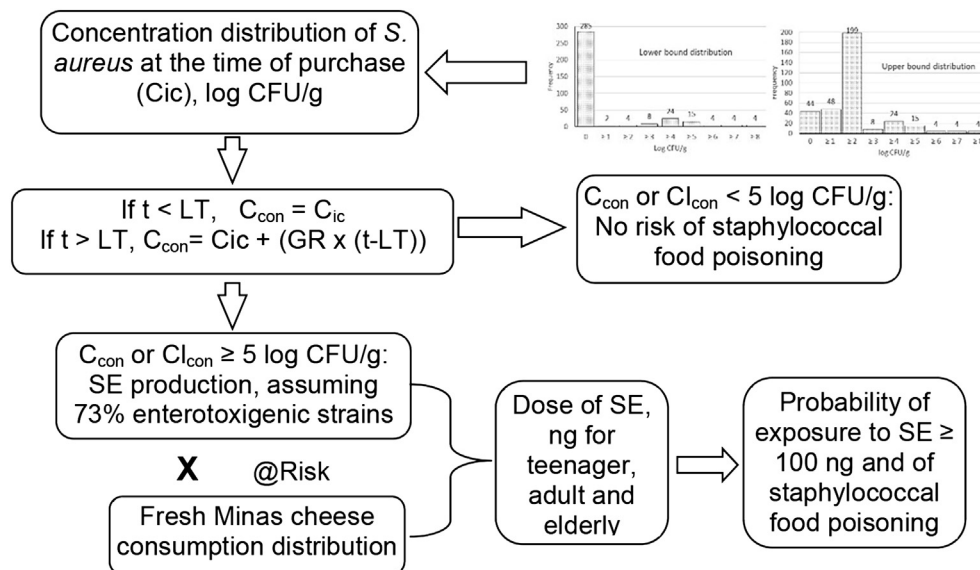
Different values of household storage temperature, pH, and % NaCl were tested in the ComBase and PMP models to investigate their impact on the growth rate and lag-phase. In the ComBase model, the growth rate increased considerably with the storage temperature T (by a factor of approximately 10 from 7.5 °C to 17.5 °C), and with the pH (60–80% higher at pH 6.5 compared to pH 5), and decreased slightly (~10%) with the % NaCl (Table S1; Supplementary material). Similarly, the lag-phase time was most affected by the T and pH (inversely proportional).

The largest impact on the growth rate using the PMP model was also observed with the change in household storage temperature, with about 4–5 times increase from 10 °C to 17 °C; there was an increase of about 2X from pH 5 to pH 6.5 (Table S2; Supplementary material). The % NaCl had a higher impact on growth rate than what

Table 1

Inputs and equations for Quantitative Microbiological Risk Assessment for staphylococcus enterotoxin A (SE) in fresh Minas cheese using @Risk®.

Input/output	Variable	Equation	Description	Unit
Input	P_{sa}	$=\text{RiskDiscrete}([0,1], [0.8143,0.1857])$	<i>S. aureus</i> prevalence in cheese, lower bound	%
Input	P_{sa}	$=\text{RiskDiscrete}([0,1], [0.1257,0.8743])$	<i>S. aureus</i> prevalence in cheese, upper bound	%
Input	C_{ic}	$=\text{RiskHistogram}(\text{Concentração de } S. aureus)$	Initial concentration of <i>S. aureus</i> in cheese	Log UFC/g
–	C_{il}	$=\text{If}(P_{sa} = 0; 0; C_{ic})$	Initial level contamination of <i>S. aureus</i>	UFC/g
Input	T	$=\text{RiskHistogram}(\text{temperature distribution})$	Temperature (°C) at the household	°C
Input	t	$=\text{RiskUniform}(1;168)$	Time between purchase and consumption	hours (h)
–	GR_C	$= (0.0227 \cdot T - 0.0757)^2$	Growth rate formula (ComBase)	Log UFC/g/h
–	GR_P	$= (0.0191 \cdot T - 0.0718)^2$	Growth rate formula (PMP)	Log UFC/g/h
–	LT_C	$= 1 / (0.0414 \cdot T - 0.1379)^2$	Lag time formula (ComBase)	hours (h)
–	LT_P	$= 1 / (0.0207 \cdot T - 0.1267)^2$	Lag time formula (PMP)	hours (h)
–	MPD	Fixed, 9.6 log CFU/g	Maxim density population of <i>S. aureus</i> , PMP only	Log UFC/g
–	C_{con}	$= \text{If}(P_{sa} = 0; 0; \text{if}(t < LT; (C_{ic}); C_{ic} + (GR_{C,P} \times (t - LT_{C,P}))))$	Contamination of <i>S. aureus</i> at the time of consumption	Log UFC/g
Input	Cl_{con}	$= \text{if}(C_{con} = 0; 0; \text{if}(C_{con} < MPD; C_{con}; MPD))$	Contamination level at the time of consumption	Log UFC/g
Input	P	$= \text{RiskHistogram}(\text{portionssizedistribution})$	Portion sizes of fresh Minas cheese for teenager, adults and elderly (grams) (POF 2008/2009)	g
–	P_{tg}	Fixed, 73%	Prevalence of toxigenic genes (Arcuri et al., 2010)	%
–	Tox	$= \text{if}(Cl_{con} \geq 5; 10^{(0.9301 \cdot (Cl_{con} \cdot P_{tg}) - 6.6621)}; 0)$	Dose of SE at the time of consumption (Kim et al., 2009)	ng/g
Output	$D_{C,P}$	$= \text{RiskOutput}(“SE dose”) (P \cdot Tox)$	SE dose for teenager, adults and elderly	ng

**Fig. 1.** Schematic diagram of the model for estimating the probability of exposure to SE from the consumption of fresh Minas cheese. t = Time between purchase and consumption; LT = lag phase time; C_{con} = concentration at consumption; GR = growth rate (Fig. 2); Cl_{con} = contamination level at consumption. On the top right, the distribution of *S. aureus* in the 350 cheese samples analyzed, for the lower (left) and upper bound distribution.

was observed with ComBase (by 45% at 10 °C and pH 5). In general, the growth rates obtained by the ComBase model were higher than those obtained in the PMP, by a maximum factor of 2 at 4.5%NaCl and pH > 5 (Tables S1 and S2). Inversely, the lag-phases obtained in the ComBase (calculated using a constant physiological state of 0.5) were much lower. In the PMP, the lag-phase was given by the model, and the calculated physiological state ($=10^{(-\text{lag} \times \text{growth rate})}$) varied significantly with the storage temperature (by a factor of 6.7 from 10 to 17 °C at pH 5) and pH (by a factor of over 300 from pH 5 to 6.5 at 12.5 °C). The maximum physiological state was 0.22 (at pH 5.5 and 17 °C) (data not shown).

The data showed clearly that storage temperature was the parameter that most impacted the grow-rate of *S. aureus* in both models, similar to what was found in other studies conducted in milk (Ding et al., 2016; Heidinger et al., 2009), and it was included in the risk assessment. The linear regressions for the growth rates and lag time as a function of storage temperature were generated for each model (GR_C and GR_P and LT_C and LT_P ; Fig. 2), considering a

constant condition of 1.1% NaCl and pH 5.5, which reflect the most common salt concentration and pH for fresh Minas cheese (Carvalho et al., 2007; Ribeiro, Simões, & Jurkiewicz, 2009; Rocha et al., 2006).

3.3. Exposure assessment of staphylococci enterotoxin (SE) in fresh Minas cheese

Table 1 describes the input parameters for the exposure assessment estimated by @Risk. Two doses (lower and upper bound) were estimated in this study, D_C using the equations generated by the ComBase (GR_C e LT_C), and D_P using the equations generated by the PMP model (GR_P e LT_P). Fig. 1 outlines the rationale used to estimate the model output (dose D) for the Brazilian populations based on their consumption pattern.

Table 2 shows the doses of SE estimated by both models (lower and upper bounds). The highest doses were obtained for teenagers and adults using the ComBase model (mean of 0.09 and 0.4 ng for

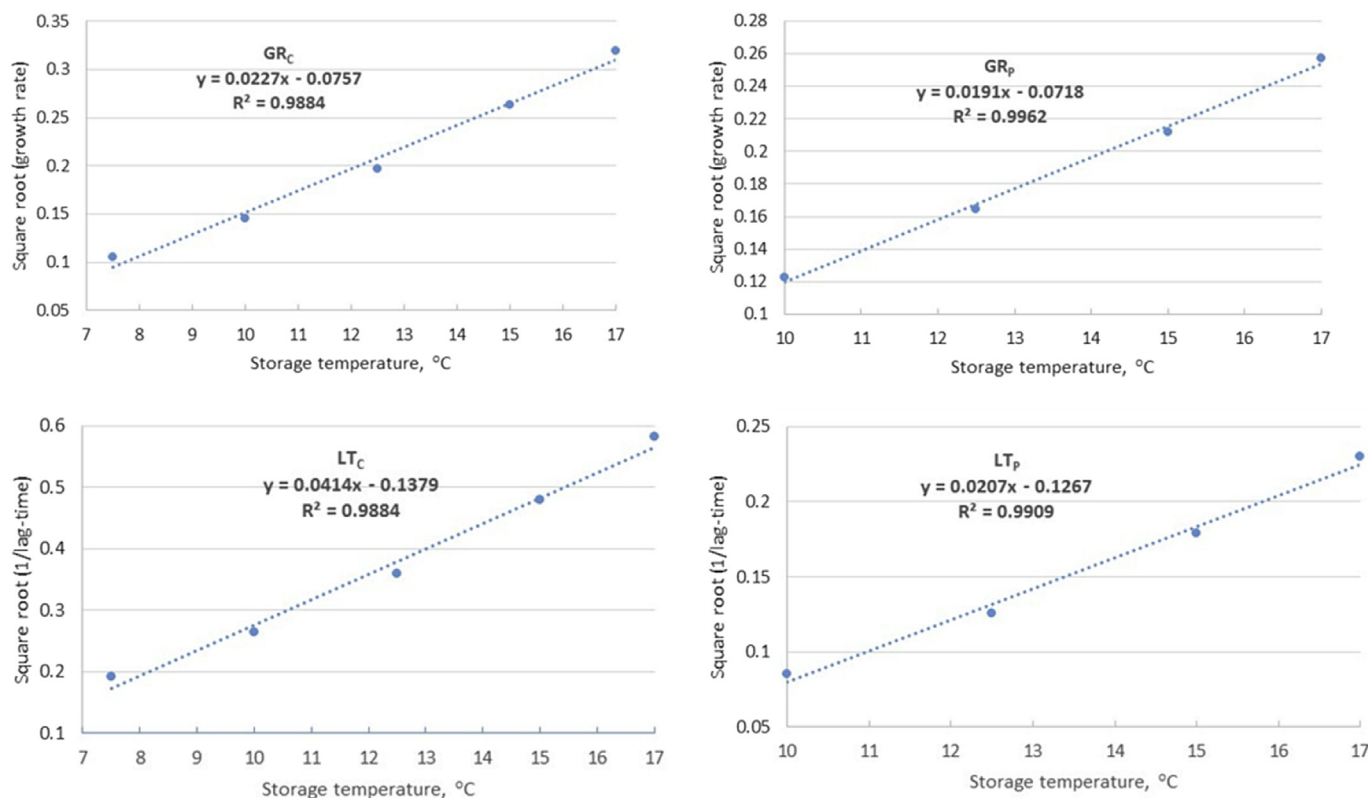


Fig. 2. *Staphylococcus aureus* growth and lag-time formulas obtained for the ComBase and PMP models, at pH 5.5 and 1.1%NaCl.

the lower and upper bound estimations, respectively), and were approximately 20% higher than the doses obtained using the PMP.

Sensitivity analyses showed that, in both models, the *S. aureus* initial concentration (C_{ic}) was the parameter that most impacted the output (Fig. 3). For the lower bound estimates, the prevalence (P_{sa}) of positive sample was the second parameter that most affected the results, what was expected as it is very low in this approach (18.57%). For the upper bound estimates, the second most important parameter was either the storage temperature or the portion of cheese consumed, depending on the model and the population (Fig. 3).

For adults, the toxigenic dose (100 ng) was exceeded at the 99.988th percentile and at 99.950th percentile of exposure in the

lower and upper bound, respectively when the ComBase model was used (Table 2). For elderly, the risk of exceedance occurred at 99.995th P and 99.976th P (lower and upper bound, respectively).

4. Discussion

Due to the limitation of data, CPS level was used as a proxy for *S. aureus*, a conservative but valid approach for microbiological risk assessment. *S. aureus* is one of the most frequent bacteria isolated from Brazilian Minas cheese (André et al., 2008; Araújo, Pagliarini, Queiroz, & Freitas-Almeida, 2002), although the number of studies that investigated the presence of enterotoxigenic strains is very limited. Sabioni, Hirooka, and Souza (1988) reported that 80% of the ten *S. aureus* strains isolated from the Minas cheese involved in a foodborne outbreak in the state of Minas Gerais produced SEA; 20% of the isolates produced SEB, 30% produced SED and 10% produced SEE. In the investigation conducted by Carmo et al. (2002), three pieces of the homemade Minas cheese involved in a foodborne outbreak in the same Brazilian state contained SEA, SEB, and/or SEC. The *S. aureus* strains isolated from Minas cheese samples collected in an outbreak that occurred in the Federal District in the context of the present study, however, were not toxigenic. In the most extensive study available (Arcuri et al., 2010), 73% of the 70 strains isolated from 12 brands of Minas frescal cheese made from pasteurized milk were enterotoxigenic. This prevalence was used in the QMRA conducted in the present study (Table 1).

Veras et al. (2008) found that all 15 CPS isolates obtained from dairy products involved in food poisoning outbreaks in the country were SE producers. The authors also found that among eight coagulase-negative staphylococci (CNS) isolates, five were genotypically and phenotypically enterotoxigenic, three of them SE producers. The occurrence of SE genes in CNS isolates has been described by other authors (Blaiotta et al., 2004; Podkowik, Park,

Table 2

Staphylococcus enterotoxin (SE) intake (ng) (lower bound) from the consumption of fresh Minas cheese servings and risk estimation, based on ComBase and PMP growth modules, considering pH 5.5 and 1.1%NaCl.

	ComBase			PMP		
	Adult	Teenager	Elderly	Adult	Teenager	Elderly
Lower bound						
Maximum	208.3	198.6	125.5	187.6	190.3	130.5
Mean	0.09	0.09	0.07	0.05	0.05	0.04
SD	2.28	2.13	1.85	1.48	1.64	1.34
Variance	5.2	4.52	3.43	2.19	2.67	1.78
$P_{\geq 100 \text{ ng}}$	99.988	99.996	99.995	99.996	99.995	99.997
Upper bound						
Maximum	216.5	198.7	205.9	254.5	199.5	154.3
Mean	0.40	0.41	0.33	0.26	0.27	0.22
SD	4.79	4.65	3.99	3.64	3.50	3.06
Variance	22.9	21.6	15.9	13.2	12.21	9.33
$P_{\geq 100 \text{ ng}}$	99.950	99.966	99.976	99.976	99.981	99.981

P = percentile at exposure ≥ 100 ng; SD = standard deviation.

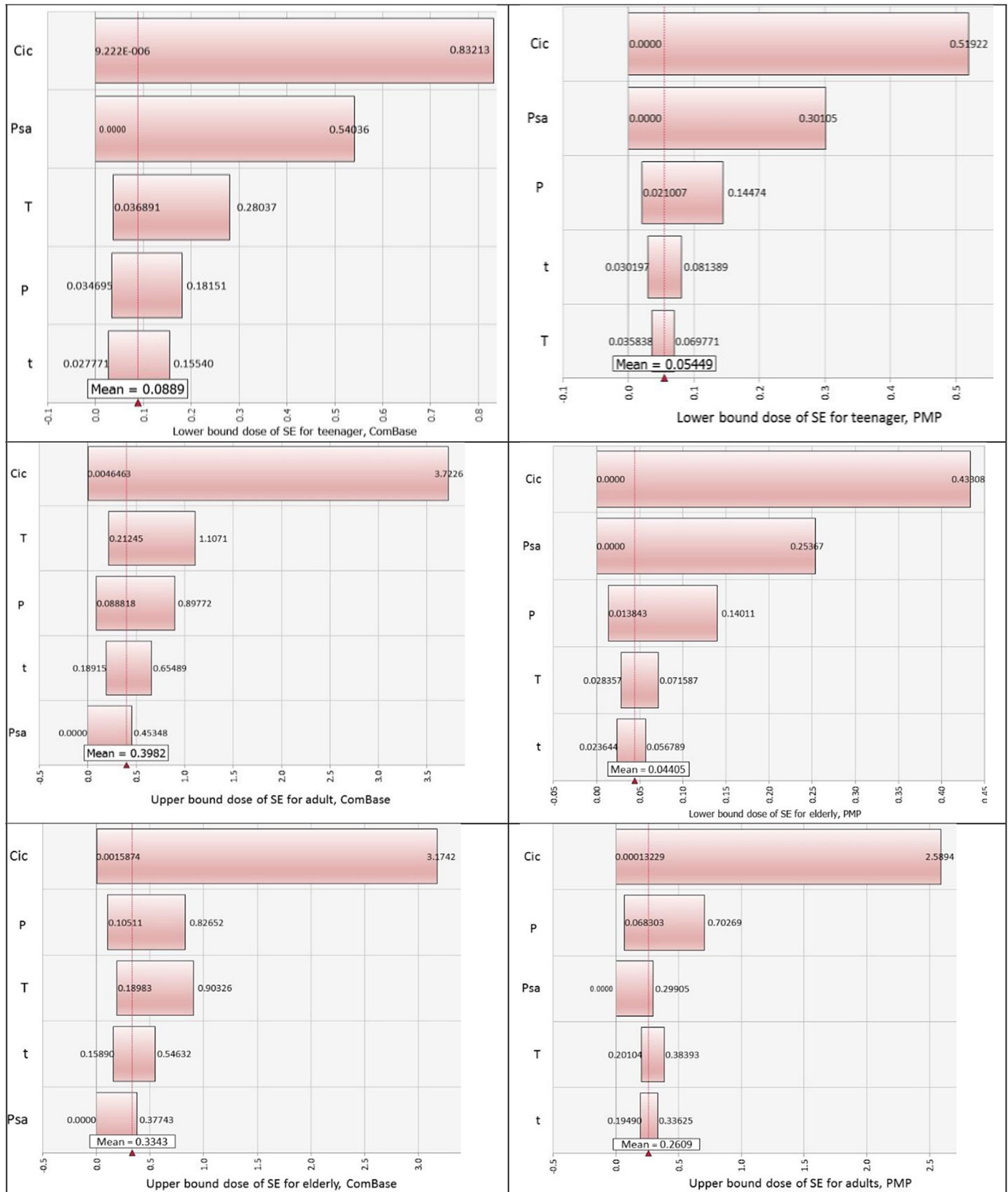


Fig. 3. Impact of the variables in the mean dose output of staphylococcus enterotoxin (SE) estimated by @Risk using the ComBase and the PMP models. C_{ic} = Initial concentration of *S. aureus* in cheese; P_{sa} = *S. aureus* prevalence; P = fresh Minas cheese portion; t = Time between purchase and consumption; T = household storage temperature.

Seo, Bystron, & Bania, 2013). This information indicates that some of the fresh Minas cheese samples that were not CPS may contain

strains of SE producers.

The risk of exposure to SE depends directly on the dose that is

considered toxic, which is determined from clinical and epidemiological studies, and may vary among populations and age groups (Larkin, Carman, Krakauer, & Stiles, 2009). The toxic dose considered in this study (100 ng; FDA, 2012), may be conservative for health young individuals, but some studies have considered a dose even lower (20 ng; Kim et al. 2009; Makita, Desissa, Teklu, Zewde, & Grace, 2012). Heidinger et al. (2009) considered a SEA toxic dose of 94 ng, based on a large outbreak of staphylococcal food poisoning involving chocolate milk that occurred in the United States of America. The authors, also using the ComBase and PMP models and @risk, found that this dose may be exceeded at the 99.99 percentile after the consumption of raw milk by the American population, and concluded that raw milk servings do not pose a significant risk from SEA intoxication. Lee, Kim, Choi, and Yoon (2015) found the maximum probability of illness per person per day in processed or natural cheese in the order of 10^{-6} , much lower than what we found for fresh Minas cheese in the present (in the order of 10^{-4}).

The exposure doses estimated by the models were mainly affected by the initial concentration of *S. aureus*, similar to what was found in other studies (Heidinger et al., 2009; Kim et al. 2009; Lindqvist, Sylvén, & Vågsholm, 2002). This result indicates the importance of good manufacturing practices and proper storage conditions at the selling point for fresh Minas cheese, parameters that were not evaluated in this study due to lack of data. Inspections at production plants and points of sale by health authorities, followed up by legal action, when necessary, are essential to guarantee that the products are available to consumers in good sanitary conditions.

This study has some limitations that need to be considered. First, the number of samples analyzed (350) was relatively small, and the data may not reflect the overall contamination rate of *S. aureus* in Minas cheese. Secondly, the enumeration was not registered in most cases, but the results were expressed as below 3, 10 or 100 CFU/g, zero, or absent. Two approaches were taken to investigate the impact of these values on the intake estimation: inputting a value of 1 CFU/g to all censored and zero/absent data to estimate a lower bound intake, or replacing the censoring data for the limit indicated (3, 10 or 100) to estimate the upper bound intake. The upper bound intake was at least four fold higher than the lower bound intake, and most likely overestimates the exposure, mainly due to the higher number of values reported as <100 CFU/g (56% of all values). On the other hand, the lower bound intake may have underestimated the exposure and the risks as no differentiation between artificial (below a certain limit) and true zeroes was done (Duarte, Stockmarr, & Nauta, 2015).

Furthermore, *S. aureus* was not actually analyzed in any of the samples, and CPS results were used to infer bacterial levels, with a fixed prevalence of toxigenic strains, what most likely led to an overestimation of the risks. This limitation shows the importance of having good monitoring data for a sound risk assessment that can be used by public health authorities and food producers (WHO/FAO, 2009). The Brazilian monitoring programs should include the analysis of *S. aureus* in the milk and milk products samples, and the identification of possible toxin producing strains. This is even more relevant from the fact that CNS samples can be toxin producers, as discussed previously.

The ComBase and PMP models used in this study have limitations as they do not consider the competitive microbiota and the expression of enterotoxigenic genes under different conditions, nor the presence of food additives to prevent bacteria growth. Furthermore, they rely on a reduced set of conditions to estimate growth (temperature, %NaCl and pH), and do not consider the level of lactic acid in bacterial growth, which is essential when milk and milk products are the food of concern (Rosengren, Lindblad, & Lindqvist, 2013). Hence, it is possible that the growth rates were

overestimated, which is nevertheless acceptable for conservative risk models. Furthermore, the PMP model sets a maximum population density (MPD) of 9.6 log CFU/g that can be reached with the growth model, which was also the maximum CPS count found in the fresh Minas cheese samples. Using this model, no increase in the concentration was predicted even if the sample with the highest count had been stored for a period longer than the lag phase. It is important to point out that some limitations and uncertainties identified in this study were also reported in other studies (Heidinger et al., 2009; Lindqvist et al., 2002; Mürmann, Corbellini, Collor, & Cardoso, 2011; Sant'Ana, Franco, & Schaffner, 2014; Sobrinho, Destro, Bernadette Franco, & Landgraf, 2014). These limitations, however, should not prevent researchers from conducting QMRA studies.

Few QMRA studies have been conducted in Brazil (Mürmann et al., 2011 - *Salmonella* in pork sausage; Sant'Ana et al., 2014 - *Salmonella* and *Listeria monocytogenes* in ready-to-eat leafy vegetables; Sobrinho et al., 2014 - *Vibrio parahaemolyticus* in raw oysters), and studies conducted in other countries are also limited, mainly on *S. aureus* in milk products. The percentile of concern in risk assessment studies is generally a management decision, but an exceedance of the toxigenic dose at the 99.9th percentile of exposure or lower may be considered to represent a health concern for staphylococcal intoxication (Heidinger et al., 2009).

In conclusion, this study indicated that the consumption of fresh Minas cheese by the Brazilian population is most likely safe. The limitations identified and discussed above should be addressed by governmental authorities and food producers in their surveillance programs to allow the improvement of the assessment.

Appendix A. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.foodcont.2016.08.046>.

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