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Update on a model to describe *Salmonella* spp. population reduction in Italian salami during production and high-pressure processing

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Dear Dr. Hopkins,

I am submitting this letter in order to update our recently published article entitled “Reduction of *Salmonella* spp. populations in Italian salami during production process and high-pressure processing treatment: validation of processes to export to the U.S.” in Meat Science Journal (Bonilauri et al., 2019).

In our previous work ten enterprises producing Italian salami were involved, and 20 challenge tests on different fermented sausages were performed in order to assess the reduction of *Salmonella* spp. during manufacturing and High-Pressure Processing (HPP). A linear regression model was used to describe the *Salmonella* spp. decay: the total *Salmonella* spp. reduction during manufacturing was significantly associated with pH at the end of acidification/drying process,  $a_w$  at the end of the seasoning period, the duration of seasoning and the caliber of salami. HPP showed to further reduce the *Salmonella* spp. count with an efficacy that resulted inversely associated with  $a_w$  of salami at the end of seasoning. The objective of 5-Log reduction was always reached after manufacturing and HPP in all the tested salami types.

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We further studied the linear regression model used to describe the *Salmonella* spp. decay during the production process and high-pressure processing treatment. To do so, 19 additional challenge tests on fermented sausages were performed, adding three enterprises to the ten previously involved, and six supplementary salami types to the nine already tested (Pepperoni small caliber, Flattened pepperoni, Milano small caliber, Hungarian, Hungarian small caliber and Garlic). The results of the new tests confirmed that *Salmonella* spp. Log Colony Forming Units (CFU)/g reduction was associated with pH at the end of the acidification/drying process,  $a_w$  at the end of the seasoning period, the duration of seasoning, and the caliber of salami in the univariate analysis. In the multivariate analysis pH at the end of the acidification/drying process,  $a_w$  at the end of the seasoning period and the duration of seasoning remained significant ( $p < 0.001$ ) with a coefficient of determination ( $R^2 = 0.485$ ).

An updated model that better encompasses all variables was obtained:

$$\Delta s = 33.06725 + 0.0193796 T_2 - 2.4978 \text{ pH}_2 - 20.211 a_{w3}$$

where:  $\text{pH}_2$  = pH at the end of the acidification/drying process;  $a_{w3}$  =  $a_w$  at the end of the seasoning period and  $T_2$  = the duration of seasoning.

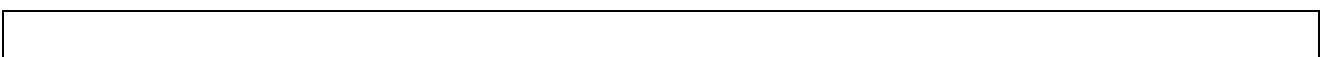
Compared to the previous model, the new one describes more accurately the decay of *Salmonella* spp. Log reduction within the previous and the latest challenge tests. Overall, the production process showed a mean *Salmonella* spp. reduction of 2.477 Log CFU/g (SD 0.898), the mean decay predicted by the updated model was 2.476 Log CFU/g (SD 0.622), with a mean of residuals of 0.002.

A validation of the updated model was performed using eight challenge tests further enrolled in 2020 (Table 1) following the methods proposed by Ross (1996), calculating the Accuracy factor (Af) and the Bias factor (Bf) of the model. The Af indicates to what extent the prediction of the model differs from the observed data, thus providing an indication of the goodness of fit. The closer the value of Af is to 1, the better the model makes the description of the experimental data. The Bf indicates whether the predicted data are numerically above or below the experimental data, thus marking a structural deviation of the model. The model showed to be safe (Bf  $< 0.95$ ) with an Af of 1.11 which means that the predictions are, on average, within 11% of the observation.

As previously observed HPP was able to further reduce *Salmonella* spp. count, mean 3.561 Log CFU/g (SD 0.637); in the new model, HPP efficacy was inversely associated with  $a_w$  at the end of seasoning, confirming the previous observations, but without giving any better description.

Processing, in combination with HPP, led to a 5 Log *Salmonella* spp. decay.

This new model, being more precise than the previous (mean of residuals of the first model applied to all the dataset  $0.251 \gg 0.002$  mean of residuals with updated model), shows to be safe with a good accuracy in



validation step, representing a better tool for enterprises and Authorities for evaluating the efficacy of the processes and HPP to reduce *Salmonella* spp. load.

Figure 1: Predictions of the updated model of Bonilauri et al. (2019) for *Salmonella* spp. describing the *Salmonella* spp. decay ( $\Delta_s$ ) observed at the end of the seasoning period in Italian salami compared to independent *Salmonella* spp. decay data obtained in late 2020. The diagonal line is the line of identity. Points above this line represent decay predictions smaller than the observed and are hence considered ‘fail-safe’ predictions. Conversely, points below the line of identity are ‘fail-dangerous’ predictions. The data are detailed in Table 1.

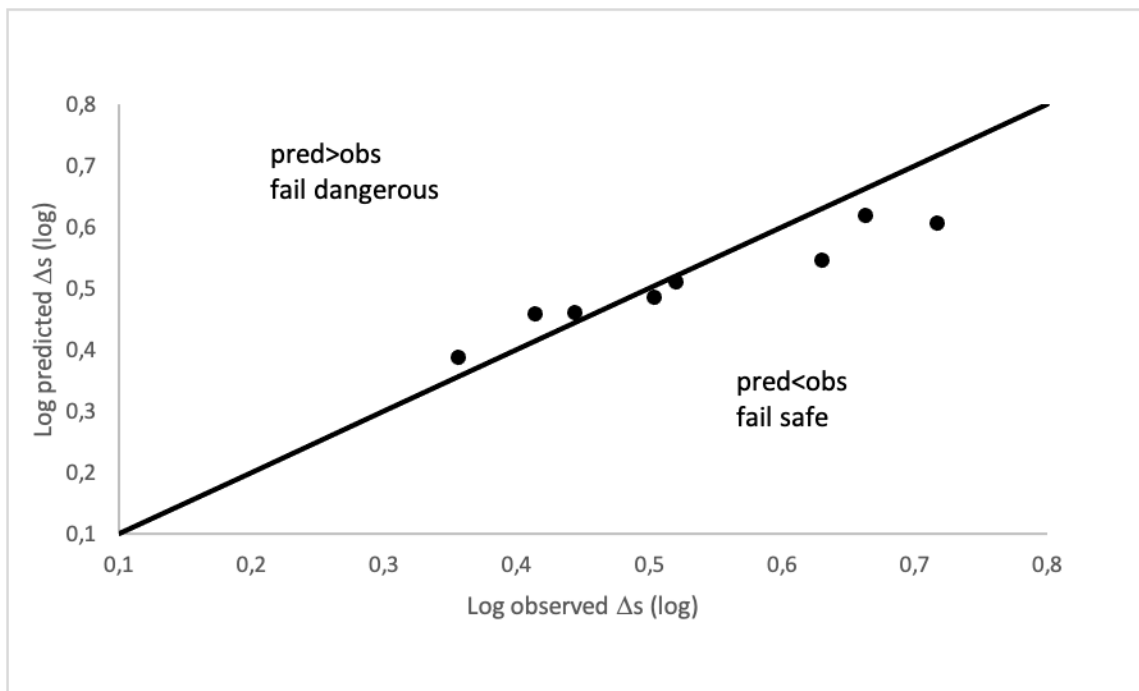
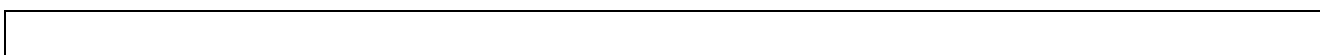


Table 1: validation of the predictive model updated from Bonilauri et al. 2019 with the calculation of bias and accuracy factors

Salami type	Variables				Observed $\Delta_s$ (log)	Predicted $\Delta_s$ (log)	Predicted/ Observed	Log (pred/obs)	Absolute value
	pH	Aw3	Ts (days)	2					
Felino salt	low	4.89	0.9	30	3.31	3.24	0.980	-0.009	0.009



Pepperoni	4.88	0.923	35	2.78	2.90	1.043	0.018	0.018
Pepperoni	4.93	0.904	30	3.19	3.06	0.960	-0.018	0.018
Pepperoni large caliber	4.86	0.912	80	5.21	4.04	0.776	-0.110	0.110
Pepperoni	4.91	0.92	35	2.59	2.89	1.114	0.047	0.047
Milano	4.44	0.924	45	4.6	4.17	0.907	-0.042	0.042
Smoked Hungarian	4.79	0.929	62	4.27	3.53	0.826	-0.083	0.083
Pepperoni small caliber	4.98	0.918	19	2.27	2.44	1.075	0.032	0.032
						Bf*	-0.021	0.045
							0.95	
						Af*	1.11	

\*Af = Accuracy factor; \*Bf = Bias factor

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