51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

EVALUATING THE USE OF PREDICTIVE MODELS FOR CRITICAL LIMIT VALIDATION IN RAW PORK PRODUCTS

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Key Words: Salmonella, Escherichia coli O157:H7, Staphylococcus aureus, raw pork, predictive model

Introduction

Under the Hazard Analysis Critical Control Point (HACCP) system, Critical Control Points (CCP's) identified by the processor are steps in a food process at which control can be applied in order to prevent, eliminate, or reduce the likelihood of a food safety hazard. (USDA 1996). The major CCP in HACCP plans for raw meat and poultry products is often the production step at which the product is the warmest or a later cooling step. Critical limits at a CCP must be met to ensure control of hazards previously identified by the processor. For raw meat and poultry products, these hazards are the growth of the infectious pathogens *Escherichia coli* O157:H7 and *Salmonella* spp., and excessive growth of *Staphylococcus aureus* which may result in the production of heat-stable enterotoxin. Ideally, critical limits associated with these pathogens would be validated using experimental challenge studies, but this is not feasible for most very small processors. An alternative validation method would be to analyze processing parameters using computer-generated predictive models of pathogen behavior such as the USDA Pathogen Modeling Program (PMP 7.0; Agricultural Research Service, Eastern Regional Research Center, Wyndmoor, PA). A shortcoming of this method is that it is based on isothermal growth conditions using laboratory media and not an actual meat product. The goal of our project was to develop a model based on isothermal growth conditions in raw pork and to evaluate the resulting model with a series of challenge studies.

Objectives

Develop a predictive model for pathogen growth during the short-term temperature abuse of pork products using lag-times experimentally determined in isothermal studies using ground pork

Conduct pathogen challenge studies and evaluate safety predictions derived from our resulting model and PMP 7.0.

Methodology

Preparation of Inoculum. Five-strain cocktails of *Escherichia coli* O157:H7, *Salmonella* spp., and *Staphylococcus aureus* were used to inoculate meat products. Inoculation cultures (20-24 h, 35°C) were prepared in Brain Heart Infusion broth (BHIB; Difco, Becton-Dickinson, Sparks, MD) from working culture plates. To prepare multi-strain cocktails, the five strains for each pathogen were combined into a

50 mL centrifuge tube (Falcon Brand, Fisher Scientific, Itasca, IL) and centrifuged for 12 minutes at 5,000 x g. The supernatant in each tube was decanted and the pellet was re-suspended in 25 ml of Butterfield's phosphate diluent (BPD, Nelson Jameson, Marshfield, WI). From each of the 5-strain pathogen cocktails, 10 mL were combined into another 50 mL centrifuge tube, creating a 15-strain, 3-pathogen cocktail which was then diluted 1:100 in BPD to make the final inoculum. Separate inocula were prepared for each trial as described above.

Isothermal Studies. Ground pork was used in developing a conservative isothermal-based model (IBM) for predicting growth in a wide range of pork-containing products, such as bacon, frankfurters, and sausage, that may contain inhibitory ingredients such as spice, salt, and cure agents. Boneless pork loin roasts were obtained from a local retailer and ground (4 mm grinding plate). Ground product was then stored at -20 ºC until 24 h before use when the product was thawed at 5ºC. Thawed ground pork (25 g) was packed into 50 mL centrifuge tubes and a hole ~ 3-4 mm in diameter was placed in the center of the pork using a sterile bent plastic spreader (Daigger, Vernon Hills, IL) to a depth of ~ 2 cm for inoculation. Isothermal studies were conducted at 2.8 ºC (5 ºF) intervals ranging from 21 - 49ºC (70 – 120 ºF). Meat-packed centrifuge tubes were held at a specific temperature until a calibrated temperature probe (K-type, Dickson, Inc.; Chicago, IL) indicated that the center of the meat reached the test temperature. Each sample was then inoculated in the hole created in the center of the meat mass with 100 µL of the pathogen cocktail. Samplings were at time-zero and hourly thereafter for three concurrent trials with one tube per trial sampled at each time.

Challenge Study. To evaluate the IBM, pathogen behavior in ground hot-boned pork and spiced pork sausage containing ground hot-boned pork (obtained from a processor) was evaluated during cooling and abusive storage. The ground hot-boned pork had pH of 6.1 and contained 4.3% water-phase salt, and spiced pork sausage had pH of 6.2 and contained 4.6% water-phase salt. Preparation and inoculation of product was done identically to the isothermal studies except one trial was done with two tubes prepared for each sampling time. Two cooling curves, differing in cooling rate, were developed for each product (Table 1) to mimic commercial practice. Each product was warmed to the starting temperature, inoculated and then cooled to 3°C (34.7°F) in 7 h by gradually decreasing the temperature of an incubator. Another study with spiced pork sausage, involved abusing the product by holding it at 21°C for 17 hrs. At several time intervals throughout the cooling and holding processes, duplicate samples were analyzed for inoculum cell numbers.

Enumeration of Inoculum Organisms. For microbial analysis, the contents of a centrifuge tube were transferred to a filter sampling bag, suspended with 99 mL of BPD and stomached at medium speed for 30s. Subsequent dilutions were made in BPD and spread-plates were prepared (one plate per dilution) on Sorbitol MacConkey agar (SMAC; Oxoid, Inc., Ogdensburg, NY), XLD agar (Oxoid), and Baird-Parker agar base (B-P; Difco) with tellurite egg yolk supplement (Difco) for enumeration of *E. coli* O157:H7, *Salmonella* spp, and *Staphylococcus aureus*, respectively. Plates were incubated at 35°C for 24 h (SMAC and XLD) and 48 h (B-P). Typical colonies were counted and log CFU/sample was calculated. For challenge studies 3M™ Petrifilm™ Staph Express plates (PF-SE; 3M Microbiology, St. Paul, MN) were used to enumerate *S. aureus*. The PF-SE plates were incubated at 35°C for 24 h. Only red-to-purple colonies were observed on PF-SE plate after 24 h for all samples; these were counted as presumptive *S. aureus* and the thermonuclease disk analysis was not necessary. It has been shown that there is no significant difference between counts on

PF-SE and B-P for meat products (Ingham et al, 2003). Throughout the study, the identity of all presumptive isolates was confirmed.

Data Analysis and Model Development. From the isothermal study data, a Critical Time (CT) was defined for each pathogen at each temperature. For *Salmonella* spp. and *E. coli* O157:H7, CT was defined as lag time (time until one doubling or 0.3 log CFU increase). Because *S. aureus* is only a significant hazard under conditions allowing growth to high levels and production of enough enterotoxin to cause illness (Jay, 1992), CT for this pathogen was defined as the time until a 1.3 log CFU increase in population had occurred, i.e. lag time plus 1.0 log CFU increase. The change in population at each sampling time (relative to time-zero) for each independent trial was calculated. The mean change in log CFU/sample and standard deviation were then calculated for each sampling time for the three independent trials. Using a one-sided t-test (Snedecor and Cochran, 1980), the CT values at each test temperature were determined, and an IBM was developed to predict growth for each challenge study (Table 2). In order to use the IBM to predict pathogen behavior in ground hot-boned pork and spiced pork sausage, the temperature profile for each trial was divided into 2.8°C (5°F) intervals and the time that the temperature was within each interval was determined. In chronological order, the time for which the product was in a given temperature interval was divided by the CT for the lowest temperature in the interval. The resulting value, multiplied by 100, equaled the % of CT elapsing in the time interval. For example, if a product was between 23.8 and 21°C for 25 minutes and the CT for *S. aureus* at 21°C was 400 minutes, then the % CT for that interval would be 6.7 = [(25 / 400) x 100]. With each successive temperature interval during product cooling, the % of CT in that interval was calculated, along with the cumulative % CT. When cumulative % CT exceeded 100 for a pathogen, the IBM predicted that the process was unsafe.

For comparison, the same product temperature intervals were used in two different applications with the PMP 7.0 model. In the first application (PMP 7.0-plain) compositional values of pH 6.2, % water-phase salt of 0.5, and 0 ppm ingoing nitrite were assigned. A second application (PMP 7.0- product) used the actual product pH and % water-phase salt values. The pathogen initial level was set to 3.0 log, and the "level of concern", or LC, was 0.3 log CFU higher for *Salmonella* spp. and *E. coli* O157:H7, and 1.3 log CFU higher for *S. aureus*. When aerobic and anaerobic models existed for a pathogen, the more conservative, i.e. more likely to predict growth, aerobic model was chosen. In chronological order, the time in each temperature interval was divided by the predicted LC time, and then multiplied by 100 to result in the % of LC time. A cumulative % LC time was calculated as each temperature interval was analyzed. When this value exceeded 100 for a pathogen, the process was predicted to be unsafe.

The log CFU/sample data from the challenge studies was statistically analyzed as described above for the isothermal studies and the mean change in log CFU/sample at each sampling time was evaluated to determine whether the CT had been exceeded.

The predicted CT of IBM and the predicted LC time of the PMP 7.0-plain model for the challenge studies were evaluated by a paired t-test (Snedecor and Cochran, 1980) with a 5% significance level. When a model predicted no CT or LC time for a given trial, a value of the length of the trial plus 1 minute was used.

Results & Discussion

No significant pathogen growth occurred in either pork product during cooling (Table 3), suggesting that use of critical limits based on the cooling curves would ensure product safety. However, significant growth did occur in spiced pork sausage held at 21.1°C for 17 h. Our IBM and PMP 7.0-plain predicted growth of *Salmonella* spp. and *E. coli* O157:H7 for all cooling curves. The PMP 7.0-plain also predicted growth of *S. aureus* during slow cooling of ground hot-boned pork. Growth of all three pathogens was predicted by IBM and PMP 7.0-plain for 21°C storage of spiced pork sausage. It is clear that both IBM and PMP 7.0-plain are conservative in predicting pathogen growth. PMP 7.0-product, using the actual pH and % water-phase salt of both products, did not predict growth for the cooling curve challenge studies and agreed with experimental data. However, PMP 7.0-product failed to predict growth of *E. coli* O157:H7 and *S. aureus* which occurred in the 21°C storage experiments with spiced pork sausage. Thus, the use of this application of PMP 7.0 can be fail-dangerous and therefore inappropriate for processors to use, while our model and PMP 7.0-plain were fail-safe. IBM and PMP 7.0-plain were not significantly different in predicting CT and LC time. Thus, IBM effectively validates use of the PMP 7.0-plain model. However, processors must perform and interpret several calculations in using PMP 7.0-plain to evaluate their process safety. Further development of IBM will result in the processor only having to input time and temperature data to obtain an easily understandable output of “safe” or “not safe”.

Conclusions

In conclusion, our isothermal-based model (IBM) and the PMP 7.0-plain application of PMP 7.0 are not significantly different when applied to the cooling and holding of raw ground pork products. Both models are conservative in their predictions, which will help processors ensure a safe product. Further development of IBM will make it simple for processors to use in predicting process safety.

References

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Tables and Figures

Table 1. Incubator settings for challenge study cooling of ground hot-boned pork and spiced pork sausage.

Ground hot-boned/Rapid		Ground hot-boned/Slow		Spiced pork sausage/Rapid		Spiced pork sausage/Slow	
temp (°C)	time (min)	temp (°C)	time (min)	temp (°C)	time (min)	temp (°C)	time (min)
37.7	Start	37.7	Start	21.0	Start	21.0	Start
32.2	30	32.2	45	15.6	45	18.3	45
26.7	30	29.4	30	10.0	45	15.6	45
21.0	30	26.7	30	8.9	30	12.8	45
15.6	45	23.9	30	7.8	30	10.0	45
10.0	45	21.0	30	6.7	30	8.9	30
8.9	30	18.3	45	5.6	30	7.8	30
7.8	30	15.6	45	4.4	30	6.7	30
6.7	30	12.8	45	3.0	180	5.6	30
5.6	30	10.0	45			4.4	30
4.4	30	7.2	30			3.0	90
3.0	90	4.4	30				
		3.0	15				

Table 2. Isothermal-based model (IBM) used to predict process safety

	Time (min) at given temperature to reach Critical Limit		
Temp (°C)	*E. coli* O157:H7	*Salmonella* spp	*S. aureus*
37.8	120	240	480
35.0	120	240	480
32.2	180	240	480
29.4	180	300	480
26.6	300	300	420
23.8	300	300	720
21.0	360	420	720

Table 3. Experimental and predicted growth of *Escherichia coli* O157:H7 (EC), *Salmonella* spp. (SALM), and *Staphylococcus aureus* (SA) in cooling/warm holding of two pork products. Predicted values were obtained from our isothermal-based model (IBM), the USDA ARS PMP 7.0 model with (PMP 7.0-product) and without (PMP 7.0-plain) adjustment for actual product pH and % salt.

Pathogen Growth: - (safe; no growth) or time (min) at which growth observed/predicted

Product/Cooling	Pathogen	Experimental	IBM	PMP 7.0 plain	PMP 7.0 product
Ground hot-boned pork/Rapid	*EC*	-	274	241	-
	SALM	-	367	351	-
	SA	-	-	-	-
Ground hot-boned pork/Slow	*EC*	-	242	166	-
	SALM	-	337	312	-
	SA	-	-	362	-
Spiced pork sausage /Rapid	*EC*	-	360	396	-
	SALM	-	420	408	-
	SA	-	-	-	-
Spiced pork sausage mix/Slow	*EC*	-	360	396	-
	SALM	-	420	408	-
	SA	-	-	-	-
Spiced pork sausage /21°C hold	*EC*	660	360	396	-
	SALM	900	420	408	936
	SA	1020	720	606	-