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PREDICTIVE MODEL FOR OUTGROWTH AND GERMINATION OF *CLOSTRIDIUM PERFRINGENS* SPORES IN CURED AND UNCURED PORK HAM DURING COOLING

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Introduction

Clostridium perfringens is a ubiquitous organism normally present in a variety of meat and poultry products and frequently associated with foodborne disease outbreaks (Doyle, 2002; ICMSF, 1996; Labbe, 2000). The U.S. Centers for Disease Control and Prevention (CDC) estimates more than 248,000 cases of foodborne illness due to Cp annually in the United States (Mead et al, 1999). The major contributing factors leading to food poisoning by Cp include its ability to form heat resistant spores that survive commercial cooking operations and its very rapid growth rate at relatively high temperatures (Labbe, 2000). Germination and outgrowth of Cp spores during cooling of thermally processed meat products has been reported extensively in the literature (Doyle, 2002; Labbe, 2000, ICMSF, 1996). Predictive models for its growth in some meat systems are currently available in the literature and as electronic databases such as the USDA's Pathogen Modeling Program and ComBase (Huang, 2003; Juneja et al, 1993; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001). These predictive models are used as decision-making tools to determine potential growth of the target organism and assert product disposition. USDA-FSIS published performance standards for lethality and stabilization of meat and poultry products (USDA, 1999, 2001). Further, the agency provided guidelines as safe harbors for cooling of these products. The stabilization performance standards focus on the potential survival of sporeformers during traditional thermal processing schedules and their germination and outgrowth during cooling. The stabilization guidelines state that processed meat and poultry products be cooled from 54.5ºC (130ºF) to 26.6ºC (80ºF) within 1.5 h and from 26.6ºC (80ºF) to 4.4ºC (40ºF) within 5 h. While cured products are required to be cooled from 54.5ºC to 26.6ºC within 5 h and from 26.6ºC to 4.4ºC in 10 h. Because of the risk of Cp growth under improper cooling conditions; meat processors incorporate cooling regimes as critical control points in their HACCP plans. However, equipment malfunctions or power failures may occur in commercial processing operations and cause cooling deviations, resulting in growth of Cp. Because of this, more information is necessary on the growth characteristics of this organism subjected to different rates of cooling in commercially formulated products. expected to be more accurate in describing the behaviors of Cp in commercially processed products

Objectives

Hypothesis: Predictive models developed under commercial conditions of thermal processing and cooling will accurately describe outgrowth of Cp spores in artificially inoculated pork ham subjected to different scenarios of cooling.

Objectives: The objectives of this study were (i) to develop predictive models to describe germination and outgrowth of Cp spores in commercially formulated pork ham; and (ii) to validate these models under commercial (exponential) cooking and cooling conditions

Methodology

Bacterial cultures. Procedures outlined by Juneja et al. (1993) were followed to prepare a 3-strain-cocktail (NCTC 8238, 8239 and ATCC 10388) of enterotoxin-producing Cp strains that have been implicated in foodborne illness. Strains were selected based on the growth rate and resistance to thermal processing.

Sample preparation. Five gram portions of commercially prepared pork ham per treatment were inoculated with 0.1ml of the spore-cocktail (ca. 3.0 log CFU/g of meat), vacuum sealed, massaged and stored under refrigeration as previously described (Huang, 2003; Juneja et al, 1993; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001).

Heat activation. Samples were submerged in refrigerated water baths programmed to cook product exponentially from 4.4ºC to 71.1ºC in ca. 10 h. Temperature changes were simultaneously registered by the water bath thermometer and external data loggers.

Microbial Enumeration. Samples were diluted in buffered peptone water and pour-plated on tryptose-sulphite-cycloserine (TSC) agar plates (Oxoid). Solidified plates were overlaid with 5ml of TSC and were incubated for 24h at 37ºC in an anaerobic chamber (Huang, 2003; Juneja et al, 1993; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001).

Predictive modeling. A 3-step modeling approach was performed according to previous publications ((Huang, 2003; Juneja et al, 1993; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001):

(i) Development of primary models to describe kinetics of Cp growth under **isothermal** conditions at 10, 15, 17, 25, 30, 35, 40, 43 and 47ºC. A total of 810 samples were analyzed (2 ham formulations x 9 isotherms x 15 sampling times x 3 replications). Populations of Cp were converted to *ln* CFU/g of pork ham and fitted to the Baranyi's non-autonomous differential equation (Baranyi and Roberts, 1994; Baranyi et al, 1995; Huang, 2003) by non-linear regression (PROC NLIN procedure of SAS®).

(ii) Development of secondary models to describe the effect of **temperature changes** on growth parameters using the square-root function by non-linear regression (Huang, 2003; Juneja and Marks, 2002; Juneja et al, 1999; Juneja et al, 2001).

(iii) Solution of the first order differential equations describing the combined dynamic model with the fourth-order Runge-Kutta method (numerical technique of Matlab®).

Dynamic cooling validations. Sample bags were subjected to commercial thermal processing and cooling cycles to chill the product from 71.1 to 7.2ºC for 9, 12, 15, 18, 21 and 24 h. A total of ca. 540 samples were examined (2 ham formulations x 6 cooling cycles x 15 sampling times x 3 replications).

Results & Discussion

Very few studies on germination and outgrowth of Cp in pork products are available. This may be due to the fact that very few reported outbreaks have been associated with pork products. Low numbers of cases associated with pork products may be related to high concentrations of salt (> 3%) in pork hams and other derivatives (Zaika, 2003). Cp has been detected in the liver and fluid from the body cavity of pork carcasses as well as 100% of samples from scalding vat water (Doyle, 2002). Therefore, presence of the organism in pork products such as ham is possible and potential outgrowth during cooling must be elucidated.

Isotherms. Characteristic (sigmoid) growth curves of Cp in commercially processed pork ham were obtained in this study. No Cp growth was observed in uncured or cured ham incubated at 10 or 15ºC for up to 30 days. As previously reported (Gough and Alford, 1965), cured samples showed longer germination times when compared to non-cured counterparts thus supporting the reasoning behind the addition of curing salts to these products. It also supports the fact that there is no history of Cp diarrhea associated with cured meat products since the organism is relatively sensitive to sodium chloride and nitrite (Gibson and Roberts, 1986; ICMSF, 1996; Labbe, 2000).

Primary modeling. Three replications per temperature were used for model fitting with the Baranyi function to a sigmoidal growth curve for each isothermal evaluated. The parameters of this function allow the calculation of lag phase duration, generation times and exponential growth rate (Baranyi and Roberts, 1994; Baranyi et al, 1995; Huang, 2003).

Secondary modeling. The expanded square-root Ratkowsky model was used to fit all the values for maximum specific growth rate derived from each replication that were estimated by the Baranyi function. Maximum specific growth rate for Cp in **pork ham** subjected to thermal processing as a function of temperature can be described by Equation 1, while Equation 2 describes the model for **cured pork ham**.

Eq. 1 $$\sqrt{\mu_{\max}} = 0.0625\left(T - 9.7806\right)$$

Eq. 2 $$\sqrt{\mu_{\max}} = 0.0563\left(T - 8.8253\right)$$

Where μ_{max} is the exponential growth rate and T is the temperature (°C) as a function of time. Regression coefficients and estimated parameters for maximum and minimum temperatures for growth are also provided.

Validations. Dynamic cooking and cooling profiles obtained from a commercial processor were used to validate predictive models. Cp spores were able to germinate and grow in uncured pork ham samples from an initial population of ca. 2.91 log CFU/g by 2.09, 2.19, 3.31, 4.80 and 4.83 log CFU/g subsequent to 9, 12, 15, 18 and 21 h exponential chill rates from 54.5 to 7.2ºC, respectively. Growth observed in cured pork ham samples from an initial population of ca. 2.87 log CFU/g was 0.68, 1.46, 2.94, 3.05 and 3.41 log CFU/g subsequent to 12, 15, 18, 21 and 24h exponential cooling from 54.5 to 7.2ºC. Predicted versus observed data for the 21 h cooling cycle of uncured pork ham is displayed in Figure 1.

There are some studies that evaluated outgrowth of Cp in processed uncured pork products. Formulated pork injected with salt, phosphates and starch at commercial

levels and inoculated with Cp spores was evaluated during the cooling from 54.5 to 7.2ºC after a heat shock treatment (Thippareddi et al, 2003). Growth of ~3.5 and 4 log10 CFU/g when cooling took 18 and 21 h respectively in control samples was inhibited by the addition of organic acid salts (buffered sodium citrate and sodium diacetate) at levels higher than 1%. Slightly higher growth levels were observed in this study 4.80 and 4.83 log CFU/g for 18 and 21 h cooling; differences that may be associated with the activation method. In the Thippareddi study a heat shock protocol (constant heat of 75 ºC for 20 minutes) was used while an exponential heating method was used in this study.

There are very few studies in cured pork-products that evaluated Cp outgrowth. *C. perfringens* cook-activated spores were capable of outgrowing rapidly in cured frankfurters (50% pork, 50% beef) when incubated at 37 and 23ºC. Growth levels diminished at 15 and 12 and no growth was observed at ≤ 10ºC (Solberg and Elkin, 1970). Cp inoculated into frankfurter emulsion containing salt, curing agents and spices; survived processing at 69ºC for 30 to 48 min. Outgrowth was observed under anaerobic conditions at 23 and 37ºC but minimal or no growth was observed at < 15ºC. Similar results were observed in this study where low temperature of incubation during the isothermal analysis also inhibited growth significantly in cured samples.

Slow cooling of ham has been recognized as potentially hazardous (Doyle, 2002). *C. perfringens* inoculated in ham with curing brine survived both curing and smoking. Even a secondary heat treatment of 121ºC for 10 min only delayed but did not prevent outgrowth of spores (Gough and Alford, 1965). Zaika (2003) evaluated the influence of salt content and cooling rate on the outgrowth of Cp spores in cooked ham. Concentrations of ≥ 3.1% NaCl inhibited the growth of *C. perfringens* when hams were cooled exponentially (54.5 to 7.2ºC) for 15, 18 and 21 h. The salt levels used in this study were lower and permitted growth of the organism in both cured and uncured samples.

Conclusions

Two predictive models to estimate germination and outgrowth of C. perfringens spores in thermally processed pork ham (cured and uncured) during the entire temperature range of commercial cooling of processed meats is provided. In general, the Cp predictive model for uncured pork ham had a measure of performance ±0.56 log CFU/g, however it underpredicted (fail dangerous) when the cooling took 9, 12 and 18 h. On the other hand the model for cured pork ham had a measure of performance of ±0.86 log CFU/g and underpredicted in the cooling validations of 12 and 18h. Summarizing, both models developed perform relatively well to describe outgrowth when compared to validation experiments simulating dynamic cooling scenarios. The use of these equations in commercial settings may enable processors and regulators to evaluate the safety of commercially produced pork ham.

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Tables and Figures

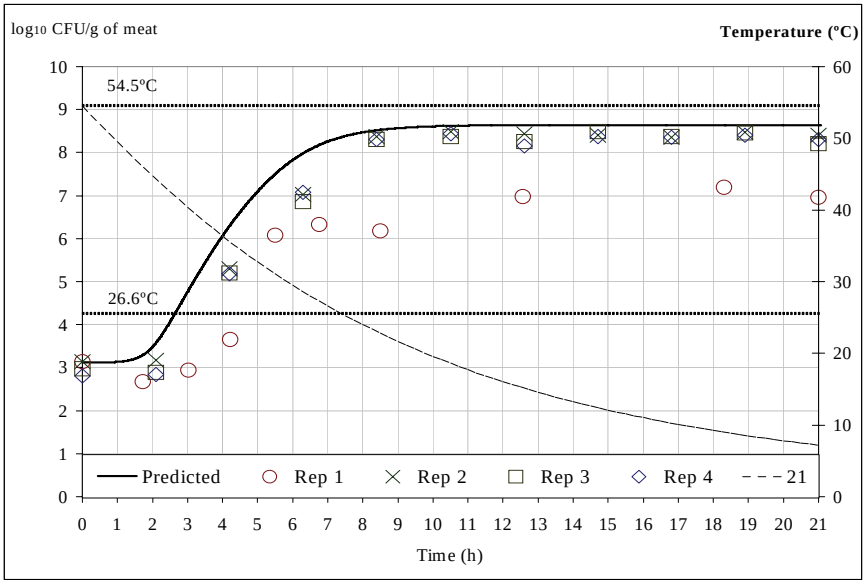

Figure 1. Experimental growth data (symbols) for heat-activated Cp spores during dynamic exponential cooling of pork ham from 54.5 to 7.2°C in 21 h. Solid line represents growth of Cp as predicted by the pork ham model. Dotted line represents the temperature profile.