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Shelf-life charts of beef according to level of bacterial contamination and storage temperature

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ABSTRACT

Shelf-life charts of beef according to bacterial contamination and storage temperature were developed via mathematical regressions of the growth curves of spoilage bacteria in beef. Additionally, bacterial contamination in retail beef specimens was investigated using culture-based bacteriological tests, PCR assays, and pyrosequencing analysis. Pathogenic bacteria were infrequently detected in the 100 retail beef specimens, but spoilage bacteria were found in all of the specimens. The populations of spoilage bacteria in retail beef specimens varied from 2.27 to 6.15 log CFU·g^{-1}. Pyrosequencing analysis of retail beef specimens suggested contamination from multiple sources, since both Lactobacillales and Pseudomonadales, which are prevalent in bovine intestine and feces and in bovine hides and beef-processing facilities, respectively, were detected. Growth of spoilage bacteria in beef at 5–25 °C was predicted via mathematical characterization of experimentally determined growth curves at 5, 10, 15, 20, and 25 °C using a modified-Gompertz function and subsequent nonlinear regressions of the growth characteristics, and then transformed to shelf-life charts according to the level of bacterial contamination and storage temperature. The bacterial contamination and bacteriological standards used to generate the shelf-life charts were 0, 1, 2, 3, 4, and 5 log CFU·g^{-1} and 5, 6, 7, and 8 log CFU·g^{-1}, respectively.

1. Introduction

Bacterial contamination of beef is an inevitable result of beef processing and handling. Contamination of beef occurs throughout slaughtering, boning and cutting, comminuting, packaging, and storage. Bacterial contaminants of beef are diverse and include non-pathogenic and pathogenic taxa. Beef is occasionally contaminated with pathogenic bacteria, such as *Escherichia coli* O157:H7, *Salmonella* spp., and *Listeria monocytogenes* (Rivera-Betancourt et al., 2004). However, most bacterial contaminants associated with beef spoilage are non-pathogenic taxa (Beuchat, 1996).

The shelf-life of beef is the time that passes before it becomes unacceptable for consumption and distribution due to the growth of spoilage bacteria. Beef harboring > 7–8 log CFU·g^{-1} spoilage bacteria is unacceptable for human consumption, both microbiologically and organoleptically (Nollet, 2012). Beef with > 7 log CFU·g^{-1} bacteria is unacceptable for international trade (ICMSF, 1986). According to the EC regulations (European Commission, 2005), the limit for spoilage bacteria in minced meat immediately after slaughtering is 5×10^6 CFU g^{-1}. Beef producers should set the shelf-lives of their products according to their intended use to manage the microbiological safety of products and prevent rejection in international trade. However, variations in bacterial growth according to storage conditions and the level of bacterial contamination hamper estimation of the shelf-life of beef (Casaburi, Piombino, Nychas, Villani, & Ercolini, 2015).

Bacterial growth follows a sigmoidal curve and can be divided into the following four phases: the lag, exponential, stationary, and death phases. The growth curve of bacteria can be characterized mathematically. The lag time (λ), specific growth rate (μ), initial population (N_0), and maximum population (N_{max}) can be calculated via regression of the curve to a mathematical model, such as a modified-Gompertz function (Shimoni & Labuza, 2000). The λ, μ, and N_{max} at a temperature (T) can be predicted via regression analyses of plots of λ, μ, and N_{max} versus T to appropriate nonlinear mathematical functions (Bruckner, Albrecht, Petersen, & Kreyenschmidt, 2013). Growth curves with different N_0 values versus storage times (t) can be replotted by application of predicted values of N_0, λ, μ, and N_{max} to the equation (e.g., a modified-Gompertz function) used for mathematical characterization of the

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growth curve, which enables estimation of the time required for a bacterial population to reach a certain size (Bruckner et al., 2013; Oscar, 2005). Therefore, growth curves of spoilage bacteria in beef at different T values facilitate estimation of the time required for spoilage bacteria in beef with different N_0 and T values to reach the bacteriological standard. Replotted growth curves of spoilage bacteria in beef can be transformed to shelf-life curves according to N_0 and T. Such beef shelf-life charts enable estimation of the shelf-lives of beef products.

This study aimed to develop shelf-life charts of beef for estimation of beef shelf-life at different N_0 and T values. To develop beef shelf-life charts, the growth characteristics (λ, μ, and N_{max}) of spoilage bacteria in beef at 5, 10, 15, 20, and 25 °C were calculated via regressions using a modified-Gompertz function. The λ, μ, and N_{max} values were predicted via nonlinear regressions using exponential decay, exponential growth, and logistic functions, and growth curves of spoilage bacteria in beef at different N_0 and T values were replotted by applying the predicted growth characteristics to a modified-Gompertz function. The replotted curves were transformed to charts of the time required for populations of spoilage bacteria in beef to reach 5, 6, 7, and 8 log CFU·g^{-1}. Additionally, bacterial contamination of retail beef specimens was investigated because it influences the bacteriological safety and shelf-life of beef. Total aerobic bacteria, coliform bacteria, and *E. coli* were enumerated in 100 retail beef specimens. The presence of *E. coli* O157:H7, *L. monocytogenes*, and *Salmonella* spp. in the beef specimens was also evaluated. To detect bacterial taxa associated with beef spoilage, the bacterial populations in retail beef specimens were subjected to pyrosequencing analysis.

2. Materials and methods

2.1. Mathematical predictions of the time required for spoilage bacteria in beef to reach bacteriological standards

Fresh beef purchased from a local market was divided into 10-g specimens. The specimens were stored in aseptic bags under aerobic conditions at 5, 10, 15, 20, and 25 °C. Triplicate specimens were sampled at appropriate time intervals and homogenized in 90 mL of saline solution. Homogenates were plated on Petrifilm aerobic count plates (PAC; 3M Com., St. Paul, MN, USA), and incubated at 35 °C for 24–48 h; colonies on PAC were then enumerated (KFDA., 2013; Linton, Eisel, & Muriana, 1997).

Growth curves of spoilage bacteria in beef at 5, 10, 15, 20, and 25 °C were regressed to a modified-Gompertz function (Eq. (1)) using the GraphPad Prism software v. 4.03 (GraphPad Software, San Diego, CA, USA), which enables calculation of λ, μ, N_0, and N_{max} values. The λ, μ, and N_{max} values at 5, 10, 15, 20, and 25 °C obtained from the regression were replotted versus T, and regressed with exponential decay (Eq. (2)), exponential growth (Eq. (3)), and logistic (Eq. (4)) functions using the SigmaPlot software v. 12.0 (Systat Software Inc., Richmond, CA, USA).

$$N = A \cdot \exp(-\exp((\mu \cdot e \cdot (\lambda - t)/A + 1))) \quad (1)$$

where N is the logarithm of a bacterial population at time (t) and A is N_{max}/N_0.

$$\lambda = \lambda_0 + a_\lambda \cdot \exp(-b_\lambda \cdot T) \quad (2)$$

where λ_0, a_λ, and b_λ are the regression constants.

$$\mu = \mu + a_\mu \cdot \exp(b_\mu \cdot T) \quad (3)$$

where μ_0, a_μ, and b_μ are the regression constants.

$$N_{max} = {N_{max}}^0 + a_m \Big/ \left[1 + (T/T_0)^{b_m}\right] \quad (4)$$

where ${N_{max}}^0$, a_m, and b_m are the regression constants.

The λ, μ, and N_{max} values at T of 5–25 °C were computed according to the regression equations and applied to a modified-Gompertz function to estimate the time ($t_E^{N_l}$) required for spoilage bacteria in beef to reach the bacteriological standards of interest (N_l; N_l = 5, 6, 7, and 8 log CFU·g^{-1}), if N_0 was 0, 1, 2, 3, 4, or 5 log CFU·g^{-1}. Based on this, the $t_E^{N_l}$ versus T according to N_l and N_0 were plotted to generate beef shelf-life charts. To improve their readability, the scales of the left and right y-axes of the shelf-life charts were in days and hours, respectively.

2.2. Bacterial contamination of retail beef

Dices of fresh beef (approximately 1 × 1 × 1 cm), delivered to retail markets on the day of or the day before collection, were aseptically collected from six randomly selected retail markets in northern Seoul, South Korea, in June 2014. A total of 100 beef specimens were collected for bacteriological tests. Among these 100 specimens, 66 were domestic and 34 were imported from Australia. Immediately after being transported to the laboratory, the specimens were divided into four 10-g pieces and transferred to aseptic plastic bags.

Ninety milliliters of saline solution was added to one of the four 10-g specimens in aseptic plastic bags and homogenized. The homogenates were diluted and plated on a PAC plate, Petrifilm coliform count plate (PCC; 3M Com), and Petrifilm *E. coli*/coliform plate (PEC; 3M Com.) for enumeration of total aerobic bacteria, coliform bacteria, and *E. coli*, respectively. Colonies of total aerobic bacteria, coliform bacteria, and *E. coli* formed on PAC, PCC, and PEC after incubation at 35 °C for 24–48 h were enumerated as described in the Korea Food Standard Codex (KFDA., 2013).

To detect *E. coli* O157:H7, 90 mL of modified tryptone soya broth (mTSB; Oxoid Ltd.) containing novobiocin (10 mg L^{-1}; Sigma-Aldrich Co., St. Louis, MO, USA) (mTSB + N) was added to the second 10-g beef specimen, which was then homogenized and enriched via incubation at 37 °C for 24 h. The enriched culture was plated on tellurite-cefixime sorbitol MacConkey agar (TCSMAC; Oxoid Ltd.) and incubated at 37 °C for 24 h. White colonies on TSCMAC agar plates were regarded as presumptive *E. coli* O157:H7 and collected using an inoculation loop, and streaked on tryptone bile X-glucuronide agar (TBX; Oxoid Ltd.). After incubation at 37 °C for 24 h, typical *E. coli* O157:H7 colonies exhibiting a blue-green color on TBX agar plates were subjected to polymerase chain reaction (PCR) assay.

To enrich *L. monocytogenes*, the third 10-g beef specimen was incubated in 90 mL of UVM-modified *Listeria* enrichment broth (UVM; Oxoid Ltd.) at 37 °C for 24 h. Then, 0.1 mL of cultured UVM broth was transferred into 10 mL of secondary enrichment broth (Fraser *Listeria* broth, FB; Oxoid Ltd.) and cultured at 37 °C for 24 h. A sterile cotton swab was soaked in cultured FB and streaked on Oxford agar (Oxoid Ltd.). After incubation at 30 °C for 24–48 h, black colonies on the plates were sampled and subjected to PCR assay.

Salmonella spp. were detected in a manner similar to that used for *L. monocytogenes* using the final 10-g beef specimen. The primary and secondary enrichment broths were peptone water and Rappaport-Vassiliadis broth (Oxoid Ltd.), respectively. The secondary enrichment culture was plated on MacConkey agar (MAC; Oxoid Ltd.) and xylose-lysine-deoxycholate agar (XLD; Oxoid Ltd.) and incubated at 37 °C for 24 h. Colonies with no color on MAC agar plates and colonies with black centers on XLD agar plates were