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Predictive modeling of bacterial growth in ready-to-use salted napa cabbage (*Brassica pekinensis*) at different storage temperatures

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ABSTRACT

The objectives of the current study were to investigate the fate of microbial indicators [aerobic plate counts (APC), total coliforms (TC), and lactic acid bacteria (LAB)] in commercial salted napa cabbages during storage conditions at different temperatures (5, 22, and 30 °C, for up to 72 h) and to develop a predictive growth model using the modified Gompertz equation to determine shelf life. Microbial population sizes (initial log CFU g^{-1}: APC, 5.1; TC, 3.0; LAB, 3.7) remained stable at 5 °C, but rapidly increased by 2–4 log CFU g^{-1} within 12 h at 22 and 30 °C; furthermore, the pH of salted napa cabbages decreased significantly ($P < 0.05$: initial pH 6.3; final pH 4.1–4.4) due to LAB fermentation. The pH showed a negative correlation with all bacterial groups and did not prevent the growth of TC during storage. According to the modified Gompertz model ($R^2 \geqq 0.97$), the highest μ_{max} was observed for LAB at 30 °C [0.61 log CFU h^{-1}], while the lowest was noted for TC at 5 °C [0.04 log CFU h^{-1}]. Shelf-life was determined using APC (7.7 log CFU g^{-1}) and LAB (6.0 log CFU g^{-1}) limits; the microbiological acceptability period of salted napa cabbage was predicted to be 12.6 and 9.3 h at 22 and 30 °C, respectively. Thus, consumers should use the product within 12 h of storage at room temperature (more quickly in the summer (9 h)), or store it in a refrigerator. The presented research proposes a shelf-life modeling of commercial salted napa cabbages, which may be used as a scientific basis for product quality control and issuing appropriate guidance for consumer use at home.

1. Introduction

Currently, consumers are spending less time in the kitchen due to changing lifestyles and are therefore seeking solutions to maximize their free time (Agriculture and Agri-Food Canada. International Markets Bureau, 2010). This social change is mirrored by an increasing trend towards minimally-processed ready-to-eat (RTE) or ready-to-use (RTU) products and is associated with the convenience of having fresh produce involving minimum preparation time before consumption (Ragaert et al., 2007). Salted napa cabbage (*Brassica rapa* sub sp. *pekinensis*) is a representative RTU product that is used as a main ingredient of *kimchi* or as a side-dish in the Asia-Pacific region [*suan cai* (China) and *tsukemono* (Japan)] (Solomon, 2014). Since the preparation of salted napa cabbage is both time and labor intensive due to trimming, salting, and washing of heavy napa cabbages (Park, 1997), consumers prefer to use commercial salted napa cabbages when preparing dishes such as *kimchi* at home.

To the best of our knowledge, despite of the increased consumption of salted napa cabbage, a comprehensive study of bacterial growth and shelf life modeling for this vegetable has never been reported. Salted napa cabbage is generally distributed without any additional decontamination treatment; thus, the storage conditions could play an important role in preventing bacterial growth. Nevertheless, according to a survey of consumer behavior related to the use of commercial salted napa cabbage at home (n = 514), consumers tended to store the vegetable at room temperature (85.4%), with the maximum storage time being 3 d (7.0%) (Rhee and Park, 2015). Since consumers use salted napa cabbage with no further processing at home, and no guidance is provided with respect to its consumption and storage, it is essential to identify an appropriate storage time and temperature.

Level of microorganisms in salted napa cabbage is an important factor for its storage, because the bacterial indicators including total

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aerobic bacteria, total coliforms (TC), and lactic acid bacteria (LAB) are closely associated with its quality. Aerobic Plate Counts (APC) of 3–6 log CFU g^{-1} are frequently observed in RTE or RTU vegetables (Nguyen-the and Carlin, 1994), and the maximum acceptable APC level in a RTU vegetable is determined at the end of the microbiological shelf life at 5×10^7 CFU/g (= 7.7 log CFU g^{-1}) (Ministere de l'Economie des Finances et du Budget, 1988). TC is the representative hygiene indicator that is useful for assessing whether good hygiene practices are being implemented (Kornacki and Johnson, 2001). Lactic acid bacteria (LAB) generally constitute a fraction (2–4 log CFU g^{-1}) of the raw vegetable microbiota (Buckenhüskes et al., 1997), and spoilage of minimally-processed vegetables is observed when the LAB counts exceed 6 log CFU g^{-1} (García-Gimeno and Zurera-Cosano, 1997). As LAB counts increase during *kimchi* fermentation, a high LAB population is not accepted in salted napa cabbage.

Consequently, the objectives of this study were: (a) to investigate the fate of these microbial indicators (APC, TC, and LAB) in salted napa cabbage during storage at different temperatures (i.e., conditions that mimic consumer behavior); (b) to evaluate the relationship between variations in pH and bacterial counts during storage; and (c) to develop a predictive growth model using the modified Gompertz equation (Zwietering et al., 1991) to estimate shelf life.

2. Materials and methods

2.1. Sample preparation

Salted napa cabbages used in this study were purchased from five manufacturers (A–E) and directly transported to a laboratory at Korea University (Seoul, Korea). Salted napa cabbages (Fig. S1) were cut into four pieces (ca. 500 g each) with a sterile knife on a cutting board. Six portions of the salted napa cabbage samples (3.0 kg in total) were placed in polyethylene plastic bags (45 × 70 cm; Hansol PS; Seoul, Korea) that are generally used for *kimchi* packaging. The samples were stored at 5 °C (the usual refrigerator temperature), 22 °C (room temperature), and 30 °C (abuse temperature) in a low-temperature incubator (VS–1203P1; Vision Scientific Co., Ltd., Daejeon, Korea). They were then collected after 0, 6, 12, 24, 48, and 72 h. These temperatures and storage times were based on the results of a consumer survey about the use of salted napa cabbages at home (Rhee and Park, 2015). Every trial with samples from each manufacturer was performed in triplicate, meaning that 15 samples were tested at each storage temperature.

2.2. Microbiological analysis

Briefly, 500 g of each sample were immediately transferred to a sterile tray and chopped with a sterilized knife. Sample portions (25 g) were transferred to stomacher bags (Circulator 400 standard bags; Seward, Worthing, UK) containing 225 ml of 0.85% sterile saline and stomached at 230 rpm for 2 min (Circulator 400; Seward). The homogenates (1 ml aliquots) were serially diluted (10-fold) in 0.85% sterile saline (9 ml). Next, 0.1 ml of each dilution was spread (in duplicate) on Plate Count Agar (PCA; Difco, Detroit, MI, USA) to grow APC, on Violet Red Bile agar (VRBA; Difco) to culture TCs, or on de Man, Rogosa and Sharpe agar (MRS; Difco) supplemented with 0.002% of bromophenol blue (Sigma Chemical Co., St. Louis, MO, USA) to culture LAB. To lower the limit of detection to 10 CFU/g, 0.2 ml of undiluted samples were also directly spread on five plates containing the above-specified media (i.e., collectively, 1 ml was plated per medium). The PCA plates were then incubated at 35 °C for 48 h, the VRBA plates at 37 °C for 24 h, and the MRS plates at 35 °C for 72 h in an atmosphere of 5% CO_2.

2.3. Measurement of distribution temperature, pH, salinity, and sugar content

The distribution temperature of every salted napa cabbage from the five manufacturers was checked using a probe thermometer immediately after receipt by the laboratory (Pocket digital thermometer A1, T9233C; DAIHAN Scientific, Seoul, Korea). The pH, sugar content, and salinity were measured in all samples. The pH of each salted napa cabbage sample during storage was monitored using a pH-meter (SevenCompact™ S220; Mettler-Toledo Inc., Columbus, OH, USA) and in accordance with the US standard method for acidified foods (21 CFR 114.90) (Code of Federal Regulations, 2016). The salinity and sugar content were analyzed as described previously, with some modifications (Heu et al., 2003), using a digital salt meter (PAL-ES2; ATAGO Co., Ltd., Tokyo, Japan) and a Brix electronic refractometer (PAL-1, Atago Co. Ltd.), respectively.

2.4. Mathematical modeling of bacterial growth

All growth curves for APC, TC, and LAB in salted napa cabbage samples at 5, 22, and 30 °C were fitted to the modified Gompertz equation, as reported previously (Zwietering et al., 1991) [Eq. (1)] and the response variables [lag phase duration (LPD) and maximal growth rate (μ_{max})] determined using the Matlab package R2016b (MathWorks, Natick, MA, USA). Robust least squares regression with least absolute residuals (LAR) was used to minimize the effects of outliers. The LAR procedure identifies a curve minimizing the absolute difference of the residuals rather than the squared differences (Ozkaya et al., 2006):

$$y(t) = y_0 + C^{*}\exp\left\{ -exp\left\{ \left[(2.7182^{*}\mu_{max})^{*}\frac{LPD - t}{C} \right] + 1 \right\} \right\} \quad (1)$$

where y(t) is the log CFU g^{-1} of bacterial counts at time t; y_0 is the initial bacterial count (log CFU g^{-1}); C is the asymptotic increase in population density (calculated as an increase in log CFU g^{-1} between time 0 and the maximum population density achieved at the stationary phase); μ_{max} is the maximal growth rate (log CFU h^{-1}); LPD is given in h, and t is the storage time (h). The coefficient of determination [R^2, Eq. (2)] and the root mean square error [RMSE, Eq. (3)] were used to evaluate the goodness of the fit and the accuracy of the estimation. R^2 is a parameter that defines the variability of the model, and the RMSE is a measure of the standard error within the estimation:

$$RMSE = \sqrt{\frac{\sum_{k=1}^{n}\left(y_k - y_k^{*}\right)^2}{N}} \quad (2)$$

$$R^2 = 1 - \left(\frac{S_{yx}^2}{S_y^2}\right) \quad (3)$$

where y is the observed value; y^{*} is the estimated value; N is the number of experimental values, and S_y and S_{yx} are the total standard deviation and the standard deviation of the estimation, respectively.

2.5. Definition of microbiological shelf life

The microbiological shelf life of salted napa cabbage was defined as the time required for the bacterial content to reach a specific value, as determined by Gompertz parameters. As there is no specific definition of the maximum acceptable microbiological