

Growth evaluation of *Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes* in fresh fruit and vegetable juices via predictive modeling

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ABSTRACT

Fresh juices are often exposed to microbial contamination due to their minimal processing, which can lead to foodborne disease. Therefore, in this study, in order to understand the behavior of foodborne pathogens (*Escherichia coli* O157:H7, *Salmonella typhimurium*, and *Listeria monocytogenes*) contained in fresh juice, the growth of the foodborne pathogens was predicted using the modified Gompertz model in six vegetable juices (beet, carrot, kale, celery, cabbage, and red cabbage) and two fruit juices (lemon and grapefruit) stored at 10 °C. Except for those of *S. typhimurium* in kale juice (maximum growth rate [GR], 0.05; lag time [LT], 118.30), the GR and LT of the foodborne pathogens were predicted to range from 0.04 to 0.08 and 6.37 to 35.48, respectively, in the vegetable juices. The performance of modified Gompertz modeling was confirmed to be in the range of 0.91–1.14 in terms of the bias factor (B_f) and 1.05 to 1.62 in terms of the accuracy factor (A_f). The predictive modeling results from this study showed that vegetable juice supported the growth of foodborne pathogens.

1. Introduction

Fresh juice is generally prepared by squeezing vegetables and fruits immediately after washing, without a sterilization process. These non-sterile fresh juices are susceptible to microbial growth and therefore can cause pathogen outbreaks (Song et al., 2007). Fruits and vegetables, which are the raw materials used in juice, may become contaminated with pathogenic microorganisms at any processing stage, from cultivation to distribution (Beuchat, 1996). In fact, pathogenic bacteria such as *Salmonella*, *Listeria monocytogenes*, *Escherichia coli* O157:H7, *Campylobacter jejuni*, *Staphylococcus aureus*, and *Bacillus cereus* have been detected in fresh, leafy vegetables such as cabbage, carrots, and celery (Beuchat, 1996). In particular, *E. coli* O157:H7, *Salmonella*, and *L. monocytogenes* are the most common foodborne pathogens, and cases of infection caused by the consumption of fresh produce have been reported. *E. coli* O157:H7 infections contracted from spinach and romaine lettuce have been reported in Canada (Alegbeleye, Singleton, & Sant'Ana, 2018), and *Salmonella* outbreaks caused by the consumption of infected fruits and vegetables have occurred in North America, Europe, and Australia (Dyda, Nguyen, Chughtai, & Macintyre, 2020). In 2010, five people in Texas died of listeriosis contracted from diced celery (Gaul et al., 2013). Therefore, controlling these pathogens is key for

preventing outbreaks caused by fresh produce consumption. Although there have been few reports of outbreaks originating from vegetable juices, non-sterile vegetable juices are associated with a high risk of foodborne disease because the raw materials may be contaminated with pathogens.

A microbial predictive model is a mathematical analysis tool for analyzing and predicting the growth, survival, and death of microorganisms present in food; describing the behavior of the microorganisms; and performing quantitative assessments of microbial risk (Kowalik, Lobacz, Zulewska, & Dec 2018). These predictive models can help determine the shelf-life of food (Yoon, Bae, & Lee, 2015). Although there have been many studies on the growth of pathogens in whole or sliced fruits and vegetables (Gullian-Klanian & Sánchez-Solis, 2018; Lokere, Maslowska-Corker, Wardt, & Wijtzes, 2016; Yoon et al., 2014) and in fruit juices (Mutaku, Erku, & Ashenafi, 2005; Sharma, Beuchat, Doyle, & Chen, 2001), a limited number of studies have investigated the growth of pathogens in fresh vegetable juices. In addition, fresh fruit and vegetable juices are generally stored and distributed under refrigeration, so the growth of pathogens at these temperatures must be assessed. Therefore, this study was conducted to investigate the growth of *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* in various fresh fruit and vegetable juices stored at 10 °C and to predict the growth of pathogens

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using the modified Gompertz model. The goodness of fit of the predictive modeling equations was verified using the bias factor (B_f) and accuracy factor (A_f).

2. Materials and methods

2.1. Bacterial strains

E. coli O157:H7 ATCC (43895, 35150, and 43889), *S. typhimurium* ATCC (DT104, 19585, and 43971), and *L. monocytogenes* ATCC (19114, 19115, and 7644) were obtained from the bacterial culture collection of Chung-Ang University (Anseong-si, Republic of Korea). Stock cultures of these organisms were stored in 1 mL aliquots of tryptic soy broth (TSB; Difco, Becton Dickinson, Sparks, MD, USA) containing 60% glycerol at -80°C until use. *E. coli* O157:H7 ATCC (43895, 35150, and 43889) and *S. typhimurium* ATCC (DT104, 19585, and 43971) were grown in TSB (Difco), and *L. monocytogenes* ATCC (19114, 19115, and 7644) was grown in TSB supplemented with 0.6% yeast extract (TSBYE; Difco) at 37°C for 24 h. After 24 h of incubation, 1 mL of each bacterial culture was collected and centrifuged at 13,000 rpm for 2 min. The centrifuged cell fraction was resuspended in 0.2% peptone water (PW, Difco) to generate a cocktail.

2.2. Preparation of fruit and vegetable juices

Fruits and vegetables were purchased from retail stores in Gyeonggi-do, Republic of Korea. The fruits and vegetables were washed with tap water, dried, peeled, cut into 3×3 cm pieces, and then juiced with a juicer (H-200 Series, Hurom, Seoul, Republic of Korea). The juice was filtered through a filter bag (3 M, Seoul, Republic of Korea), and then 10 mL samples were collected in sterilized 15 mL conical tubes (Corning, NY, USA).

2.3. pH, titratable acidity (TA), and total soluble solid (TSS)

2.3.1. pH

The pH values of the juice samples were measured using a pH meter (SK-650PH, Sato Keirtoki Co., Tokyo, Japan). The pH meter was calibrated with three standard buffer solutions of pH 4.0, 7.0, and 10.0 at room temperature. Juice samples (10.0 mL) were placed in a beaker, and their pH was measured in triplicate at room temperature.

2.3.2. TA

To measure TA, 10 mL samples of fresh fruit and vegetable juice filtered using a filter bag (3 M) was placed in a beaker, 0.2 mL of 1% phenolphthalein solution (DUKSAN GENERAL SCIENCE, Seoul, Republic of Korea) was added, and titrated with 0.1 N NaOH (DUKSAN GENERAL SCIENCE). The TA was calculated using the following equation:

$$\text{TA (\%)} = (V \times N \times \text{acid milliequivalent weight factor} \times 100) / m$$

where V is the volume of NaOH (mL), N is the normality of NaOH, and m is the mass of the juice sample.

2.3.3. TSS

TSS was measured using a digital refractometer (pal-1, ATAGO, Tokyo, Japan). The digital refractometer was calibrated at room temperature using distilled water. Fresh fruit and vegetable juices were filtered through a filter bag (3 M), collected 1 mL, and measured at room temperature. The results were expressed in terms of $^\circ\text{Brix}$.

2.4. Bacterial inoculation and enumeration

On a clean bench at room temperature, 10 mL aliquots of fresh fruit and vegetable juices filtered through a filter bag (3 M) were placed in

Table 1

Physicochemical analysis of lemon, grapefruit, beet, carrot, kale, celery, cabbage, and red cabbage juices.

Fruit and vegetable juices	Physicochemical property		
	pH	% acid (TA)	$^\circ\text{Brix}$
Lemon	2.33 ± 0.23	4.23 ± 0.19	7.45 ± 0.35
Grapefruit	3.21 ± 0.14	0.66 ± 0.44	11.16 ± 0.90
Beet	5.89 ± 0.21	0.09 ± 0.02	9.68 ± 2.23
Carrot	6.08 ± 0.31	0.15 ± 0.01	8.08 ± 0.47
Kale	5.71 ± 0.17	0.30 ± 0.05	5.82 ± 1.16
Celery	5.60 ± 0.15	0.13 ± 0.02	4.06 ± 0.63
Cabbage	5.95 ± 0.18	0.10 ± 0.04	5.06 ± 0.15
Red cabbage	6.02 ± 0.31	0.05 ± 0.02	6.22 ± 0.81

sterile 15 mL conical tubes (Corning) and inoculated with 0.1 mL of the bacterial culture solution cocktail (final concentration 10^{6-7} CFU/mL). The juice samples were stored at 10°C for 144 h, and the number of bacteria was determined at 0, 12, 24, 48, 72, 96, 120, and 144 h. For bacterial enumeration, 1 mL samples of fresh fruit and vegetable juice were serially diluted with 0.2% PW (Difco). *E. coli* O157:H7 was spread on sorbitol MacConkey agar (SMAC; Oxoid, Basingstoke, Hampshire, England), *S. typhimurium* was spread on xylose lysine deoxycholate agar (XLD; Difco), and *L. monocytogenes* was spread on Oxford Agar Base (OAB; Difco) by 0.1 mL. The bacteria were then cultured at 37°C for 24–48 h.

2.5. Data analyses

2.5.1. Growth curves

Growth curves of pathogenic bacteria in fresh fruit and vegetable juices stored at 10°C for 144 h were generated using Sigma Plot (Sigma Plot, version 10.0, Systat Software, Germany). The average value obtained from the three replicated experiments was used.

2.5.2. Modified Gompertz modeling

To obtain reliable estimates of the lag time (LT) and growth rate (GR), all data were fitted to the modified Gompertz model (Prism, version 4.2, GraphPad Software; San Diego, CA, USA) used in the present study (Yoon et al., 2014):

$$Y = N_0 + C \times \exp(-\exp [(2.718 \times \text{mue}/C) \times (\text{Lag}-X) + 1])$$

where Y is the log of the number of cells (log CFU/mL), X is the incubation time, N_0 is the initial cell number, C is the difference between the initial and final cell numbers, mue is the GR value, and Lag corresponds to the lag phase duration before growth. The coefficient of determination (R^2) was calculated using Prism (GraphPad; USA).

2.5.3. Validation of predictive modeling

The performance of the modified Gompertz model was validated in terms of B_f and A_f . The equations for determining B_f and A_f , based on Ross (1996), are as follows:

$$B_f = 10^{(\sum \log(GT_{\text{predicted}}/GT_{\text{observed}})/n)}$$

$$A_f = 10^{(\sum |\log(GT_{\text{predicted}}/GT_{\text{observed}})|/n)}$$

where B_f indicates the degree to which the observed value is above or below the line of equivalence, and A_f represents the closeness of the predicted value to the observed value on average, as the average of the distances between each point and the line of equivalence (Park, Seo, & Ha, 2007).

3. Results and discussion

3.1. Physico-chemical properties

In our previous study (Lee et al., 2021) evaluating the microbial

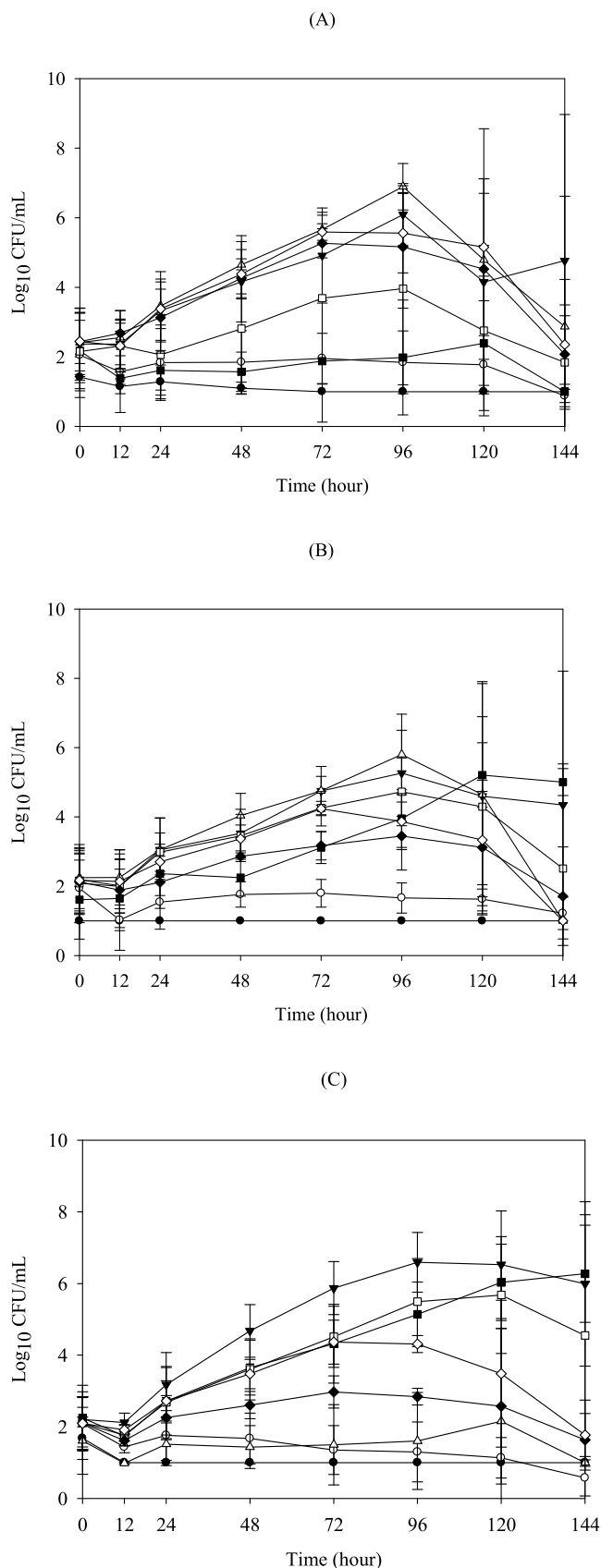


Fig. 1. Growth curves of (a) *E. coli* O157:H7, (b) *S. typhimurium*, and (c) *L. monocytogenes* in (●) lemon, (○) grapefruit, (▼) beet, (△) carrot, (■) kale, (□) celery, (◆) cabbage, and (◇) red cabbage juices stored at 10 °C for 7 d.

quality of fresh juices in Korea, juice samples in which foodborne pathogens were detected or in which the amount of total aerobic bacteria was greater than 5 log CFU/mL were defined as highly contaminated samples. Six vegetables (beet, carrot, kale, celery, cabbage, and red cabbage) and two fruits (lemon and grapefruit) were among the raw materials of the highly contaminated samples. Table 1 shows the pH, TA, and TSS of fruit and vegetable juices. All six vegetable juices had a pH above 5.60, while the two fruit juices had a pH below 3.21. The TA value is obtained by titrating the total acid content of food with an alkaline standard solution and is generally expected to be inversely proportional to pH. However, in some of the juices analyzed in this study, the pH and TA were not inversely proportional. TSS differed according to the type of fruit or vegetable; grapefruit had the highest score (11.16 ± 0.90 °Brix), and celery had the lowest score (4.06 ± 0.63 °Brix).

3.2. Growth curves

Fig. 1 shows the growth curves of foodborne pathogens observed in fresh fruit and vegetable juices stored at 10 °C for 7 days. In carrot, beet, red cabbage, cabbage, and celery juice with a pH of 5.5 or higher, the *E. coli* O157:H7 populations increased continuously but then decreased after 96 h of storage (Fig. 1A). The *E. coli* O157:H7 populations increased most rapidly in carrot and beet juice. However, in carrot juice, the population decreased after 96 h of storage, whereas in beet juice, the population was maintained at 4.77 log CFU/mL until 144 h of storage. In lemon juice and grapefruit juice, the *E. coli* O157:H7 populations tended to decrease throughout storage, and after storage for 144 h, they were below the detection limit (1.00 log CFU/mL). Although the pH of kale juice was as high as 5.71 ± 0.17 , kale juice exhibited a bacterial growth trend similar to that of lemon juice and grapefruit juice.

The *S. typhimurium* population in carrot juice increased sharply but then decreased after 96 h of storage (Fig. 1B). However, in kale and beet juice, the *S. typhimurium* populations increased continuously and then remained relatively constant until the end of storage (5.01 and 4.35 log CFU/mL in kale and beet juice, respectively). In lemon juice, the population of *S. typhimurium* decreased below the detection limit immediately after inoculation, and in grapefruit juice, the population displayed a tendency to decrease throughout the storage period.

The *L. monocytogenes* populations increased in kale, beet, celery, and red cabbage juice during storage (Fig. 1C). While the *L. monocytogenes* population in red cabbage juice decreased after 96 h, that in kale juice continued to increase for 7 days after storage, reaching 6.27 log CFU/mL (initial value, 2.24 log CFU/mL) at the end of storage. The population of *L. monocytogenes* in cabbage juice was maintained at a similar level during the initial level during the storage period. Grapefruit juice and lemon juice showed similar results for the *E. coli* O157:H7 and *S. typhimurium* populations, which either exhibited a steady decline from the initial level or fell below the detection limit within 12 h of storage. However, in carrot juice, the population of *L. monocytogenes* displayed a very different trend compared to *E. coli* O157:H7 and *S. typhimurium*, hardly increasing from the initial level during the storage period.

In this study, no *E. coli* O157:H7 growth was observed in kale juice. This might be due to the phytochemicals contained in kale. A previous study showed that the phenolic fraction of kale leaf extract has antibacterial activity against various Gram-positive and Gram-negative bacteria; for example, the glycoside phenolic fraction exerts an inhibitory effect on *E. coli* ATCC 35218 (Ayaz et al., 2008). Additionally, no *L. monocytogenes* growth was observed in carrot juice in the present study. Similarly, Noriega et al. (2010) reported that when carrot juice was inoculated with 10^7 CFU/mL of *L. monocytogenes* and stored at 4 °C, the bacterial population decreased below the detection limit (1×10^1 CFU/mL) after 1 day. This may be due to the anti-listerial activity of carrots; Nguyen-the and Lund (1991) reported that when sliced carrots were inoculated with *L. monocytogenes* and stored at 4 °C, the bacterial population decreased from 4.8 log CFU/g to less than 1.6 log CFU/g after 90 min. Moreover, Lokerse et al. (2016) reported that when 10 g of

Table 2

Growth parameters of pathogens in various fruit and vegetable juices determined via modified Gompertz modeling.

Fruit and vegetable juices	Pathogens	Parameters of modified Gompertz equation ^a		
		GR	LT	R ²
Lemon	<i>E. coli</i> O157:H7	NA ^b	NA	NA
	<i>S. typhimurium</i>	NA	NA	NA
	<i>L. monocytogenes</i>	NA	NA	NA
Grapefruit	<i>E. coli</i> O157:H7	NA	NA	NA
	<i>S. typhimurium</i>	NA	NA	NA
	<i>L. monocytogenes</i>	NA	NA	NA
Beet	<i>E. coli</i> O157:H7	0.06	10.73	0.82
	<i>S. typhimurium</i>	0.05	12.24	0.91
	<i>L. monocytogenes</i>	0.08	14.01	0.98
Carrot	<i>E. coli</i> O157:H7	0.08	11.91	0.85
	<i>S. typhimurium</i>	0.06	11.91	0.92
	<i>L. monocytogenes</i>	NA	NA	NA
Kale	<i>E. coli</i> O157:H7	NA	NA	NA
	<i>S. typhimurium</i>	0.05	118.30	0.98
	<i>L. monocytogenes</i>	0.04	7.26	0.98
Celery	<i>E. coli</i> O157:H7	0.05	35.48	0.99
	<i>S. typhimurium</i>	0.04	6.37	0.95
	<i>L. monocytogenes</i>	0.05	13.77	0.98
Cabbage	<i>E. coli</i> O157:H7	0.06	12.58	0.99
	<i>S. typhimurium</i>	0.04	23.77	0.96
	<i>L. monocytogenes</i>	NA	NA	NA
Red cabbage	<i>E. coli</i> O157:H7	0.07	12.49	0.97
	<i>S. typhimurium</i>	0.05	14.14	0.88
	<i>L. monocytogenes</i>	0.06	13.88	0.89

^a GR, maximum growth rate (1/h); LT, lag time before growth (h).

^b NA, not available.

carrots was inoculated with 3.2 log CFU/mL of *L. monocytogenes* and stored at 7 °C for 10 days, no growth was observed. Our results also showed that *L. monocytogenes* did not grow in cabbage juice, possibly due to the methyl methanethiosulfinate (MMTSO) contained in cabbage. [Kyung and Fleming \(1997\)](#) reported that MMTSO had a minimum inhibition concentration of 50 ppm against *L. monocytogenes* B70 (ATCC 19115). The growth of foodborne pathogens was observed in all vegetable juices, except for *E. coli* O157:H7 in kale juice and *L. monocytogenes* in carrot and cabbage juice in the present study. No *L. monocytogenes* growth was observed in carrot juice, but *E. coli* O157:H7 grew up to 6.89 log CFU/mL during storage. In addition, [Song et al. \(2006\)](#) reported that when carrot juice and kale juice were inoculated with *S. typhimurium* at a concentration of 10⁸ CFU/mL and stored at 10 °C, the pathogen survived at a level of 6.5 log CFU/mL after 3 days of storage.

3.3. Modified Gompertz model and model suitability validation

The R² value of the modified Gompertz model was determined to range from 0.82 to 0.99 (Table 2). The R² value describes how well the model fits the data, and the closer R² is to 1, the better the fit ([Saunders, Russell, & Crabb, 2012](#)). In lemon juice and grapefruit juice, no parameters could be obtained through predictive modeling because no growth was observed for any pathogens. Modified Gompertz modeling parameters could not be obtained because *L. monocytogenes* and *E. coli* O157:H7 did not grow in carrot juice and kale juice, respectively, and *L. monocytogenes* in cabbage juice tended to retain the initial bacterial counts during storage. The shortest LT value of *E. coli* O157:H7 was

Table 3

Bias factor (B_f) and accuracy factor (A_f) of growth prediction model for the growth of pathogens in fruit and vegetable juices.

Fruit and vegetable juices	Pathogens	Parameters of modified Gompertz equation	
		B _f	A _f
Lemon	<i>E. coli</i> O157:H7	NA ^a	NA
	<i>S. typhimurium</i>	NA	NA
	<i>L. monocytogenes</i>	NA	NA
Grapefruit	<i>E. coli</i> O157:H7	NA	NA
	<i>S. typhimurium</i>	NA	NA
	<i>L. monocytogenes</i>	NA	NA
Beet	<i>E. coli</i> O157:H7	0.99	1.08
	<i>S. typhimurium</i>	0.98	1.09
	<i>L. monocytogenes</i>	0.99	1.05
Carrot	<i>E. coli</i> O157:H7	0.99	1.15
	<i>S. typhimurium</i>	1.18	1.27
	<i>L. monocytogenes</i>	NA	NA
Kale	<i>E. coli</i> O157:H7	NA	NA
	<i>S. typhimurium</i>	0.62	1.62
	<i>L. monocytogenes</i>	1.09	1.11
Celery	<i>E. coli</i> O157:H7	1.14	1.17
	<i>S. typhimurium</i>	1.08	1.13
	<i>L. monocytogenes</i>	1.07	1.09
Cabbage	<i>E. coli</i> O157:H7	1.12	1.16
	<i>S. typhimurium</i>	1.09	1.12
	<i>L. monocytogenes</i>	NA	NA
Red cabbage	<i>E. coli</i> O157:H7	1.10	1.16
	<i>S. typhimurium</i>	1.11	1.23
	<i>L. monocytogenes</i>	1.04	1.17

^a NA, not available.

observed in beet juice, at 10.73, and the shortest LT values of *L. monocytogenes* and *S. typhimurium* were observed in kale juice and celery juice, at 7.26 and 6.37, respectively.

The predictive ability of the model must be validated to determine whether the model provides a good explanation for microbial growth in food ([Zhou, Fu, Li, Cheng, & Liang, 2009](#)). In this study, the predictive ability of the model was evaluated in terms of B_f and A_f (Table 3). A developed model is generally considered suitable when the B_f value is between 0.70 and 1.15 ([Ross, Dalgaard, & Tienungoon, 2000](#)). The B_f values of the *S. typhimurium* growth prediction model for carrot juice and kale juice were 1.18 and 0.62, respectively, which were out of the acceptable range, indicating that the model was unsuitable for growth prediction. However, the B_f values of the growth prediction model for vegetable juices, except carrot and kale juice, ranged from 0.98 to 1.12, which was within the acceptable range, indicating suitability for growth prediction. The predictive model is considered less accurate as the A_f value moves away from 1 ([Enkhjargal, Min, & Yoon, 2013](#)). The A_f values obtained as parameters of the growth prediction models for *S. typhimurium* in carrot juice and kale juice were 1.27 and 1.62, respectively, indicating that the prediction model had low accuracy. However, the A_f values of beet juice ranged from 1.05 to 1.09, indicating that the accuracy of the prediction model was relatively high.

4. Conclusions

After the growth of pathogenic bacteria in six vegetable juices and two fruit juices was observed, it was concluded that no growth occurred in fruit juice with a low pH. However, bacterial growth was observed in

vegetable juices with a relatively high pH; in beet juice, *E. coli* O157:H7, *S. typhimurium*, and *L. monocytogenes* all grew and survived until 144 h after storage. The modified Gompertz model was able to predict the growth of pathogenic bacteria in fresh vegetable juices, with R^2 values ranging from 0.82 to 0.99. The accuracy of the predictive model was verified based on the values of B_f and A_f , and it was concluded that the model was suitable for predicting the microbial growth of fresh vegetable juices, except for carrot juice and kale juice. Although the results of this study and previous studies suggest that survival or growth of pathogenic bacteria is possible in vegetable juices, related studies examining different types of vegetables remain limited. Therefore, the results of this study will improve the understanding of the behavior of pathogenic bacteria in vegetable juice. The findings can also serve as primary data informing the design of processes that extend the quality period of fresh vegetable juice and prevent foodborne diseases. However, the results of this study provide limited information; therefore, further studies are needed to better understand the growth of pathogenic bacteria in various fresh vegetable juices.

CRedit authorship contribution statement

Soyul Lee: Performing the experiment, collecting data, Visualization, Writing – original draft. **Areum Han:** Preparing the, Methodology, revising materials and methods. **Jae-Hyun Yoon:** Revising the manuscript. **Sun-Young Lee:** Designing the experiment, managing the experiment, evaluating the data, and revising the manuscript.

Declaration of competing interest

The authors declare no conflict of interest.

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