

Effect of Temperature and Time on the Bacterial Community Changes and *Enterobacteriaceae* Counts Analysis for Shelf Life Estimation of Hainan Tropical Fresh-Cut Fruit Trays

Meng Zhu, Suishan Yang, Lidan Kou, Xiuting Chang, Xiaoju Luo, Zuorong Xie*

Key Laboratory of Tropical Fruits and Vegetables Quality and Safety for State Market Regulation, Hainan Institute for Food Control, Haikou, China

Email address:

zmengdream@126.com (Meng Zhu), 15208940836@163.com (Zuorong Xie)

*Corresponding author

To cite this article:

Meng Zhu, Suishan Yang, Lidan Kou, Xiuting Chang, Xiaoju Luo, Zuorong Xie. Effect of Temperature and Time on the Bacterial Community Changes and *Enterobacteriaceae* Counts Analysis for Shelf Life Estimation of Hainan Tropical Fresh-Cut Fruit Trays. *International Journal of Nutrition and Food Sciences*. Vol. 12, No. 5, 2023, pp. 158-165. doi: 10.11648/j.ijnfs.20231205.16

Received: September 20, 2023; Accepted: October 26, 2023; Published: October 31, 2023

Abstract: Storage time and temperature are key factors in the growth of disease-causing and spoilage-causing microorganisms in tropical fresh-cut fruit trays, which affect the shelf life and food safety of fruit trays. The aim of this study was to characterize the bacterial community in tropical fresh-cut fruit trays and to establish a growth model and predict the shelf life of the fruit trays by the change in the number of *Enterobacteriaceae* bacteria to facilitate the control of storage temperature and time during the trading process. The results showed that *Proteobacteria* demonstrated significant changes at different storage temperatures conditions (6, 10 and 15°C). Sensory analysis showed a loss in freshness and texture and an increase in ripeness at the three storage temperatures, with shelf life of tropical fresh-cut fruit trays being within 24 hours at 6°C and sold within 10 hours if possible at 10°C. The growth model and shelf-life prediction model with *Enterobacteriaceae* bacterial population finally yielded a theoretical shelf-life of 7.8 h at 15°C. Based on the results of the above study, fruit retailers can adjust the storage conditions and time of tropical fresh-cut fruit trays to effectively reduce the spoilage rate of fruit trays and contribute to food loss and waste at the consumer and retail levels. Meanwhile, food safety risks can be effectively reduced.

Keywords: Hainan Tropical Fresh-Cut Fruit Trays, Bacterial Community, *Enterobacteriaceae* Bacteria, Shelf-Life

1. Introduction

Ready-to-eat fruit trays are processed fruits made from fresh fruits by a series of processes such as sorting, cleaning, peeling or pitting, cutting, mixing and packaging [1]. At present, the fresh-cut fruit products in developed countries occupied most of the retail fruit market share [2]. In China, with the improvement of people's living standards, the acceleration of life rhythm and the increasing entry of delivery services into people's lives, the instant fresh-cut fruit trays industry has been favored by consumers for its convenience and nutrition, and has taken an important position in people's daily life [3, 4]. Especially in Hainan, which is known as the "fruit basket" of the whole country, Hainan tropical fresh-cut fruit trays are an indispensable part of popular consumption, usually including

tropical fruits such as mangoes, papayas, dragon fruits, pomegranates, watermelons, honeydew melons, bananas, star fruits, etc., as well as other seasonal tropical fruits.

Although fresh-cut fruit trays are convenient and easy to eat, due to the mechanical damage caused by artificial trimming, cutting and other processing, they are prone to microbial contamination and lead to decay, discoloration or spoilage [5]. The level of microbial contamination in fresh-cut fruits and vegetables is much higher than that in unprocessed one [6-8]. Hainan Island is located in the southernmost part of China, with average annual temperature of 22-26°C and high temperature and humidity. This climatic characteristic is more likely to accelerate the spoilage of local tropic fruit trays and the multiplication of microorganisms in them. Foodborne diseases caused by microbial contamination are the main factors affecting

the quality and safety of ready-to-eat fresh fruits and vegetables [9]. The current microbial contamination control of ready-to-eat fresh-cut fruits and vegetables mainly focus on pathogenic bacteria, including *Salmonella*, *Staphylococcus aureus*, *Listeria monocytogenes*, *laxative Escherichia coli* and other pathogenic bacteria to make the limit requirements, but still lack of microbial health indicators of bacteria limit value of the standard provisions, to carry out ready-to-eat fruit plate microbial spoilage bacteria and indicators of bacteria research becomes very necessary.

Putrefactive pathogenic microorganisms on ready-to-eat fruits include *Owenella*, *Staphylococcus aureus*, *Salmonella*, *Listeria monocytogenes*, *laxative Escherichia coli*, and *Pseudomonas* in the *Proteobacteria*, which have been shown to survive on a variety of fruits such as cantaloupe, dragon fruit, and papaya [10]. And common pathogenic microorganisms such as *Salmonella* and *laxative Escherichia coli* belong to the *Enterobacteriaceae* family of bacteria. The *Enterobacteriaceae* family has been used as a food hygiene indicator in Europe for many years. This family of bacteria can become a conditionally pathogenic bacteria and cause human and animal diseases when the host immunity is lowered or the bacteria are displaced to the extra-intestinal parts [11]. In recent years, there were many food-borne disease outbreaks caused by *Enterobacteriaceae* contamination of fruits and their products [12]. In addition to the infections in the US, Germany, Sweden, France and other European and American countries [13, 14]. In China, a food safety incident caused by fruit salad contaminated by *Enterobacteriaceae* bacteria also occurred in Jinan, Shandong Province in 2019 [15]. Therefore, conducting research on the proliferation characteristics of *Enterobacteriaceae* bacteria in Hainan tropical fresh-cut fruit trays and establishing a shelf-life prediction model will have a positive contribution and risk warning significance to ensure the safe supply of Hainan tropical fresh-cut fruit trays and reduce the occurrence of foodborne diseases.

This study for the first time to explore the change of bacterial community diversity in Hainan tropical ready-to-eat fruit plate under different storage temperatures, the proliferation trend of microbial health indicator *Enterobacteriaceae* bacteria, by combining the sensory changes of the fruit plate, in order to understand the type, number and proliferation pattern of microorganisms, for Hainan tropical ready-to-eat fruit plate microbial distribution and shelf-life prediction provides a theoretical basis for microbial risk control in the process of processing, storage and consumption, in order to ensure the quality of fresh-cut fruit trays and promote the healthy development of the fresh-cut fruit plate market industry.

2. Materials and Methods

2.1. Materials

The experimental materials provided are fresh fruits that are free from pest and mechanical damage, including papaya, watermelon, pomegranate, mango and dragon fruit,

purchased from a large fresh supermarket with good hygienic conditions and storage conditions in Haikou City, China.

2.2. Main Reagents and Instruments

2.2.1. Culture Medium

Buffered peptone water, violet red bile glucose agar, nutrient agar (Guangzhou Huankai Biological Co., Ltd).

2.2.2. Instrument and Equipment

HYC-610 Medical Refrigerator (Qingdao Haier Special Electrical Appliance Co., Ltd.), Constant Temperature and Humidity Incubator (Shanghai Yihang Scientific Instrument Co., Ltd.), HVA-85 Fully Automatic Vertical Pressure Steam Sterilizer (Hirayama Co., Ltd., Japan), BSA6202S Electronic Balance (Sartorius Scientific Instrument (Beijing) Co., Ltd.), LHS-150HC-I Constant Temperature and Humidity Incubator, LRH-250F Biochemical Incubator, MJ-150F-I Fungus Incubator (Shanghai Yihang Scientific Instrument Co., Ltd.), BSC-1600IIA2 Biological Safety Cabinet (Suzhou Antai Air Technology Co., Ltd.), and NovaSeq6000 Sequencer.

2.3. Method

2.3.1. Sample and Pretreatment

Clean and peeled fresh, undamaged papaya, watermelon, mango, and dragon fruit and cut into slices (pieces). Place them in a clean fruit tray, add the washed lychee and pack with 0.04mm fresh-keeping film, and each tray has the same weight, with 25 g of each type of fruit.

Three samples of prepared fruit trays were stored at three temperatures (6°C, 10°C and 15°C) and the number of coliforms was measured every 2 hours at the first 12 hours, the last measurement was taken after 24 hours. Three trays were placed at each temperature and the whole experiment was repeated three times.

2.3.2. Analysis of Bacterial Diversity

Samples of Hainan tropical fresh-cut fruit trays at different storage temperatures were left for 12 hours and 2 g sample from each temperature was equally sampled, and then tested for bacterial population by Shanghai Meiji Biological Medicine Technology Co. Ltd. Commercial reagent kits were used to extract genomic DNA from the trays, and the purity and concentration of DNA were detected by agarose gel electrophoresis. The diluted genomic DNA was used as a template to amplify the target band by PCR with 16S 338F_806R primer and ITS1F ITS2R primer, respectively, to construct a genome library [16, 17]. After the library was quantified, the sequencing data were spliced and used for analysis of bacterial diversity in Hainan tropical fresh-cut fruit trays.

2.3.3. Determination of *Enterobacteriaceae*

The determination of *Enterobacteriaceae* was conducted according to GB4789.41-2016 «Food Safety National Standard Food Microbiology Testing *Enterobacteriaceae* Test» [18].

2.3.4. Sensory Quality Evaluation

Carry out sensory evaluation according to Table 1.

Table 1. Sensory scoring criteria for fresh-cut fruit trays.

Rating	Color and luster	exture	Odor
8-10	The flesh is brightly colored and glossy	Maintain a strong organization and not let it become limp	No odor
4-7	The fruit is slightly dull in color and lacks luster	Slightly hard in texture with no obvious wilting	No obvious odor
0-3	The flesh is dull and lacks luster	Organization turns soft, rotten and oozes out juice	have an odor

2.3.5. Establishment of Dynamics Model

(i). Establishment of Dynamics Model of Intestinal Bacteria Growth

The establishment of a primary model of the growth dynamics of *Enterobacteriaceae* mainly focuses on the relationship between microbial numbers and time. The modified SGompertz model (Equation 1) was used as the primary model, and Origin 9.0 software was used to fit the growth data of *Enterobacteriaceae* on fresh-cut fruit trays at 6, 10, and 15°C, and calculate the maximum specific growth rate (μ_{max}) and lag period (λ). The modified SGompertz model is calculated using Equations 2 and 3 [19].

In the formula, N_t and N_0 are the number of bacteria at time t and the initial time, CFU/g; a is the difference between the number of microorganisms in the steady state and the inoculated bacteria; X_c is the time required to reach the relative maximum growth rate; k is the relative growth rate (slope) at time X_c ; μ_{max} is the maximum specific growth rate, lg (CFU/g)/h; λ is the lag period.

$$\lg \frac{N_t}{N_0} = a \times \exp\{-\exp[-k \times (t - X_c)]\} \quad (1)$$

$$\mu_{max} = a \times \frac{k}{e} \quad (2)$$

$$\lambda = X_c - \frac{1}{k} \quad (3)$$

(ii). Establishment of the Square Root Model

The Square Root Model (Secondary Model) is an expression of the relationship between temperature and primary model parameters; its square root (Belehradck) equation is as follows [20]. Using the first-level model parameters, the maximum specific growth rate μ_{max} and the lag phase λ of *Enterobacteriaceae* in fresh-cut fruit trays were calculated and the relationship between them and temperature was fitted using the Square Root Model (Square Root Model), using Formula (4) and (5):

$$\sqrt{\mu_{max}} = b(T - T_{min}) \quad (4)$$

$$\sqrt{\frac{1}{k}} = b(T - T_{min}) \quad (5)$$

In the model, T is the growth temperature, T_{min} is the

Table 2. Parameter indexes of bacterial genome library in different fresh-cut fruit trays.

Storage temperature	Number of effective sequences	shannon	simpson	ace	chao	coverage
6°C	51796	0.172729	0.925687	45.76638	44.6	0.999693
10°C	45583	0.178882	0.939533	53.82727	61.25	0.999616
15°C	49702	0.22333	0.939562	56.16659	51.375	0.999642

theoretical minimum temperature for *Enterobacteriaceae* growth of 6°C and b is a parameter of the model.

2.3.6. Establishing a Shelf Life Prediction Model

By fitting the experimental data of fresh-cut fruit trays stored at 6, 10 and 15°C with the SGompertz equation to describe the growth dynamics under different storage temperatures, and selecting a suitable strain as the indicator bacteria to predict the shelf life of fresh-cut fruit trays, the shelf life of fresh-cut fruit trays can be predicted by the time required for the initial bacterial number to reach the spoilage control limit (Shelf life, SL) [21].

2.3.7. Data Analysis

Data analysis and processing were carried out using SPSS 17.00 and Excel 2019 software, and plots were made using Origin9.0 software. Microbial community analysis and heat maps were plotted using Majorbio Cloud Platform. Each experiment was repeated three times.

3. Result and Analysis

3.1. Analysis of Microbial Diversity at Different Storage Temperatures

3.1.1. Bacterial Community Diversity Analysis

Based on Table 2, analysis of the Alpha diversity index of the bacterial genome of the fresh-cut fruits trays showed that the valid sequences obtained at different temperature storage were more than 198821, with coverage rate higher than 99.9%, indicating a high sequencing depth and coverage rate. The four parameters of chao, ace, shannon and Simpson were used to analyze the bacterial diversity and uniformity in the trays. The bacterial chao index was positively correlated with the incubation temperature, indicating that the higher the temperature, the faster the proliferation of bacteria grow in the sample and the higher the bacterial abundance accumulate. Shannon and Simpson index showed that the samples under three different storage temperatures had high bacterial diversity. The trend of change of these two indices was similar to that of chao index, and the bacterial community diversity and richness showed a similar trend, among which the samples stored at 15°C had the highest bacterial diversity, suggesting.

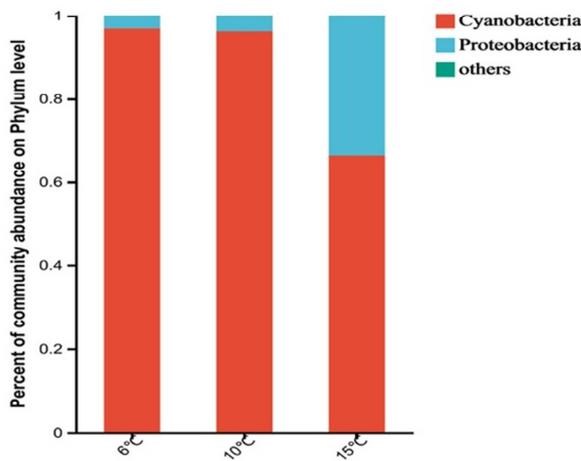
3.1.2. Relative Changes of Dominant Bacterial Community

As shown in Figure 1(A), *Proteobacteria*, *Cyanobacteria* and other unclassified bacteria were the main bacteria in the fruit trays samples. *Proteobacteria*, the largest of bacterial divisions, includes many pathogenic bacteria such as *Enterobacteriaceae*, *Salmonella*, *Cholera*, *Helicobacter pylori* and other famous species. The presence and increase of *Proteobacteria* also affects the quality of the fruit trays. When stored at 10°C, the number of *Proteobacteria* was not much different from that at 6°C. However, when the storage temperature was 15°C, the number of *Cyanobacteria* decreased significantly and the number of *Proteobacteria* increased, indicating that *Proteobacteria* are the main

microorganisms in the fresh-cut fruit trays when stored at 15°C.

Figure 1(B) shows the classification results of different storage temperatures in the fruit trays, and the proportion of *Erwiniaceae* and *Pseudomonas* is relatively high. The higher the storage temperature, the faster the number of *Erwiniaceae* and *Pseudomonas* bacteria increases, especially *Erwiniaceae* increases sharply, indicating that *Erwinia* is the dominant bacteria in the fruit trays. The *Erwiniaceae* and *Pseudomonas* is the main pathogenic microorganism that causes fruit trays deterioration [22-24]. This result shows that fresh-cut fruit trays stored at 15°C are more likely to decrease the freshness compared with 6°C and 10°C.

A



B

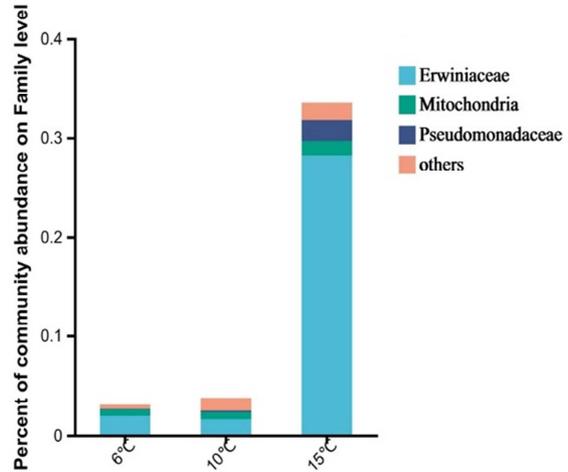


Figure 1. Analysis of community composition at the phylum level (A) and family level (B).

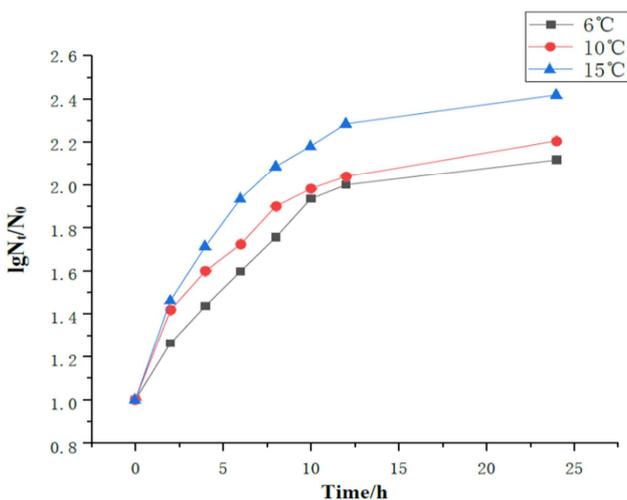


Figure 2. Changes of *Enterobacteriaceae* at different storage temperatures.

3.2. Changes of Enterobacter at the Same Storage Temperature

Enterobacteriaceae, including *Escherichia coli*, *Escherichia coli*, *Shigella*, *Salmonella*, and *Erwinia*, are

primary pathogenic bacteria that cause human will intestinal infectious diseases or food poisoning, and *Erwinia* is the main microorganism that causes bacterial softening and decay of fruits and vegetables. Temperature is an important factor affecting the survival and growth of *Enterobacteriaceae* in the environment. A study by Whitman et al [25] showed that *Enterobacteriaceae* could survive at 4°C for more than 6 months. From Figure 2, it can be seen that with the extension of storage time, the total number of *Enterobacteriaceae* showed a gradual increase. The number of *Enterobacteriaceae* in fresh-cut fruit trays increased less under storage conditions of 6°C and 10°C, while the higher the storage temperature, the faster the increase, and the number of *Enterobacteriaceae* in fresh-cut fruit trays increased the fastest under storage conditions of 15°C.

Table 3. Sensory scoring results of fresh-cut fruit trays at different storage temperatures.

Temp./°C	Time/h						
	2h	4h	6h	8h	10h	12h	24h
6°C	9.5	9	9	8.5	7.5	7	5
10°C	9	8.5	7.5	6	5.5	5	4
15°C	8	7	6	5.5	4.5	4	3

3.3. Change in Sensory Quality of Freshly Cut Fruit Trays

Sensory evaluation is an important tool for quality identification of fresh-cut fruit trays, which directly affects consumers' purchase intentions. Mechanical damage to fresh-cut fruits and vegetables after the cutting process can result in loss of juice, de-regionalization of cells and tissue structures, and loss of normal cellular functions [26]. The sensory quality scores of fresh-cut fruit trays stored at 6°C, 10°C and 15°C are shown in Table 3, and the sensory scores of fresh-cut fruit trays decrease with time. Under different storage temperatures, the color of fresh-cut fruit trays becomes dull, the tissue softens, and the time for juice to seep out varies. After 6 hours of storage at 15°C, the color of the fruit becomes dark, the tissue softens, more juice seeps out, and the decay begins; after 10 hours of storage at 10°C, water seepage softening and darkening appears; and after 24 hours of storage at 6°C, the above phenomena appear.

3.4. Dynamic Model of Growth of Enterobacteriaceae in Fresh-Cut Fruit Trays Under Different Storage Temperature

3.4.1. Establishment of First-Level Model

The growth of *Enterobacteriaceae* in fresh-cut fruit tray at different storage temperatures of 6°C, 10°C and 15°C was fitted by using Origin9.0 software with the modified SGompertz model as the primary growth model. The experimental and fitting curves show the growth trend of *Enterobacteriaceae* at

different temperatures, as shown in Figure 3.

The growth pattern of *Enterobacteriaceae* is roughly the same in three different temperatures, with a slow initial growth and slow increase in colony number, followed by a rapid growth and reproduction period, and the higher the temperature, the faster the growth. The correlation coefficient R² value calculated from the model fitting can be used to judge the fitting degree of the primary model and whether it is suitable for establishing a secondary model. The determination coefficient R² values at three different temperatures are 0.99, 0.98 and 0.99, respectively, indicating that the SGompertz model can well reflect the growth status of *Enterobacteriaceae* in fresh-cut fruit trays. The optimal primary growth dynamics model and fitting parameters are shown in Table 4 and Table 5.

According to equations (2) and (3), the maximum growth rate μ_{max} and lag rate can be calculated. As shown in Table 6, the μ_{max} of *Enterobacteriaceae* in fresh-cut fruit trays at 6°C is 0.0632116636 and at 15°C is 0.16988640610, which is much higher than that at 6°C, indicating that the growth rate of *Enterobacteriaceae* in fresh-cut fruit trays increases with the increase of temperature. At the same time, the lag rate λ decreases with the increase of temperature. Since the modeling of lag rate λ is affected by many environmental factors, such as preactivation of bacteria, initial concentration of bacteria (shorter lag phase during logarithmic period, longer lag phase during stationary and starved cell period) [27, 28], it is difficult for most predictive models to accurately predict the change of lag rate.

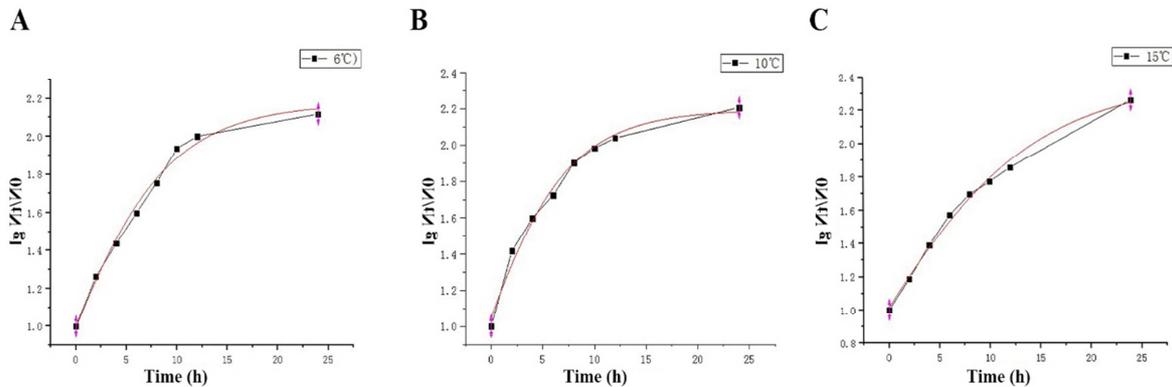


Figure 3. Growth curves of *Enterobacteriaceae* at 6°C (A), 10°C (B) and 15°C (C) in fresh-cut fruit trays fitted by SGompertz model.

Table 4. Parameters for fitting the primary growth model of *Enterobacteriaceae* in fresh-cut fruit trays.

Temp./°C	Factor			R ²
	a	Xc	k	
6	2.17408	1.41461	0.17045	0.98956
10	2.19453	1.49755	0.20345	0.98532
15	2.39274	1.40599	0.10930	0.99332

Table 5. Primary growth model of *Enterobacteriaceae* in fresh-cut fruit trays.

Temp./°C	Model of SGompertz
6	$\lg(Nt/N0) = 2.17408 \times \exp\{-\exp[-0.17045 \times (t-1.41461)]\}$
10	$\lg(Nt/N0) = 2.19453 \times \exp\{-\exp[-0.20345 \times (t-1.49755)]\}$
15	$\lg(Nt/N0) = 2.39274 \times \exp\{-\exp[-0.10930 \times (t-1.40599)]\}$

Table 6. Growth parameters at different temperatures obtained from the square root secondary model of *Enterobacteriaceae* in fresh-cut fruit trays.

Temp./°C	$\mu_{max}/\lg(\text{CFU/g/h})$	λ/h	R ²
6	0.0632116636	12.58492	0.95
10	0.1064249867	7.2548753	0.95
15	0.1698864061	6.536921	0.96

3.4.2. The Establishment of Square Root (Belehradek) Secondary Modeling

Although the one-level model can well describe the growth of *Enterobacteriaceae* at different temperatures, it cannot reflect the relationship between storage temperature and growth trend. The two-level model describes the relationship between temperature and the maximum specific growth rate

and lag time. The square root model equations (4) and (5) were used to fit the λ and μ_{\max} obtained from the one-level model into the square root two-level model; the correlation equation between temperature and the maximum specific growth rate in the temperature range of 6-15°C is $\sqrt{\mu_{\max}} = 0.0073T + 0.1521$; The correlation equation between temperature and lag time is $\sqrt{1/\lambda} = 0.0351T + 0.17$, and the determination coefficient R^2 is 0.950, indicating that the square root equation model can accurately reflect the relationship between different temperatures and the lag time of *Enterobacteriaceae* growth in fresh-cut fruit trays.

3.4.3. Construction of Shelf Life Prediction Model

(i). Determine the Limit of Corruption Control

Table 7. Changes in *Enterobacteriaceae* during storage at different temperatures in fresh-cut fruit trays.

Temp./°C	Number of <i>Enterobacteriaceae</i> (lg(CFU/g))	Sensory scores
6	2.12	5.0
10	1.90	6.0
15	1.69	5.5
Limit of Corruption Control	1.90	5.5

According to Table 7, the shelf-life control limit for spoilage of freshly cut fruit trays stored at 6-15°C that meets the commodity limit through sensory evaluation is 1.90 (lg (CFU/g)).

(ii). Constructing a Shelf Life Prediction Model

Based on the established dynamics of the growth of *Enterobacteriaceae*, the maximum number of bacteria and the initial number of bacteria to the corruption limit control amount, the shelf life of the freshly cut fruit trays stored at 6-15°C was calculated. According to the sensory and actual storage and trafficking in supermarkets, we only predicted under 15°C storage conditions. The corruption limit control of *Enterobacteriaceae* in fresh-cut fruit trays was 1.90 (lg (CFU/g)), and the maximum bacteria count was 2.25 (lg (CFU/g)) as the average of the maximum colony count at each temperature condition.

By entering the initial bacterial count obtained from the test into Equation (6), the shelf-life of Hainan tropical fresh-cut fruit tray stored at 15°C can be calculated.

$$SL = \lambda - \left[\frac{(N_{\max} - N_0)}{2.718\mu_{\max}} \times \ln \left\{ - \ln \left[\frac{(N_a - N_0)}{(N_{\max} - N_0)} \right] - 1 \right\} \right] \quad (6)$$

The shelf-life of fresh-cut fruit trays stored at 15°C was calculated to be 7.8h based on formula (6), where SL is the shelf-life of fresh-cut fruit trays, h; N_{\max} is the maximum logarithmic colony count of CFU/g; N_0 is the initial logarithmic colony count of CFU/g; μ_{\max} is the maximum specific growth rate at 15°C, d^{-1} . The measured value of shelf-life was 8.2h with a relative error of 4.87%, which is lower than 10%, indicating the accuracy of the predictive model and its practicality.

4. Conclusion

In the experiment, the dynamic changes of coliforms in fresh-cut fruit trays stored at 6°C, 10°C and 15°C were monitored regularly. The number of coliforms in fresh-cut fruit trays increased with the increase of storage temperature, while the sensory score decreased gradually. A total of 198821 valid sequences were obtained by detecting the bacterial diversity after 12h storage at the above three temperatures, and the coverage was all above 99.9%, indicating that the sequencing depth was high and the bacterial abundance and uniformity were good. The highest bacterial diversity was found in the sample stored at 15°C, and the most abundant bacteria were *Erwiniaceae* and *Pseudomonas* in *Proteobacteria*, indicating that *Erwiniaceae* and *Pseudomonas* are the key bacteria causing the spoilage and deterioration of Hainan tropical fresh-cut fruit trays. Although the number and abundance of coliforms were not reflected in the microbial diversity detection, coliforms belong to a large number of pathogenic microorganisms in *Proteobacteria*. Therefore, we determined to fit the coliforms in the modified SGompertz model, and the data of three temperatures could be described by the square root equation established in the model to predict shelf life, maximum growth rate and lag time. Based on the research on the supermarket and the actual data of the experiment, the quality of fruit trays stored at 6°C and 10°C for 24h is still within the controllable range, so this experiment only predicts the shelf life of the goods stored at 15°C, and the relative error between the predicted value and the actual value is less than 10%.

This study can provide a reference for merchants on the selling time and storage temperature of fresh-cut fruit plates in order to control the cost and reduce the waste. At the same time, it can also reduce the possibility of consumer food safety hazards due to overtime selling of fruit plates. However, this study explores the storage time of fresh-cut fruit plates at different temperatures, and subsequent work can increase the study of storage humidity for merchants and further determine the temperature and humidity control and storage time for home-made fruit plate storage, so as to facilitate the public's confirmation of the optimal period of enjoyment of homemade fresh-cut fruit plates, to ensure the nutrition, and to reduce the risk of food safety.

5. Discussion

Although fresh-cut fruit trays are quick and convenient, there are still some food safety risks. Nowadays, the shelf life of single fresh-cut fruits has been studied more in domestic and international studies [29-31], but the shelf life of fresh-cut fruit trays composed of multiple fruits has been reported less. Through research of multiple supermarkets and fruit sales stores, it is found that the storage temperature of fresh-cut fruit trays is usually 6-18°C, and the shelf life is mostly 24h. Due to the

different components of fruit trays, processing technology, storage environment and storage time, the growth trend of total bacteria, coliform bacteria and bacterial diversity will also vary with the different conditions [32-34]. Based on the characteristics of microbial growth and the actual sales situation of the fruit trays, this experiment was designed and an appropriate model was selected to predict the shelf life of fresh-cut fruit trays.

At present, due to the particularity of its processing and sales, there is no relevant microbial limit standard for ready-to-eat fruit trays issued in China. In terms of regulation of ready-to-eat fresh-cut fruits and vegetables, the implementation of two standards, «GB31652-2021 Ready-to-eat Fresh-cut fruits and Vegetables Processing Hygienic Specification» and «T/CCFAGS031-2022 Ready-to-eat Fresh-cut fruits and Vegetables Production Service Specification (Food Operators)», respectively stipulate and require the production of ready-to-eat fresh-cut fruits and vegetables enterprises and supermarkets, catering, convenience stores, fruit stores, fruit cut specialty stores, etc. food operators in the operating site. Cutting, production, distribution of ready-to-eat fresh-cut fruits and vegetables products. Therefore, this project has enriched the microbial limit data of the fresh-cut fruits and vegetables industry, and also provided a basis and guidance reference for the microbial safety and shelf life prediction of Hainan tropical fresh-cut fruit trays.

Author Contributions

Investigation, M. Z., YS. S.; Writing original draft, M. Z.; Writing review and editing, XZ. R.; Formal analysis, M. Z., YS. S. and CX. T.; Software, M. Z. and LD. K.; Data curation, M. Z., LD. K. and XJ. L. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Key Laboratory of Tropical Fruits and Vegetables Quality and Safety for Satate Market Regulation [ZZ-2023015, ZZ-2022008].

Data Availability Statement

Data is contained within the article.

Conflicts of Interest

The authors declare no conflict of interest.

Acknowledgments

We thank Yuan Wang (Institute of Genetics and Developmental Biology Chinese Academy of Sciences) and Haiyan Sun (Institute of Tropical Bioscience and Biotechnology) for the Supervision.

References

- [1] CHENG Li-lin WU Bo YUAN Hai-jun. et. Al Research Progress in Storage and Preservation Technologies of Fresh-cut Fruits and Vegetables [J]. Storage and Process, 2019, 19(01): 147-152.
- [2] BASELICE A, COLANTUONI F, DANIEL AL, et al. Trends in EU consumers' attitude towards fresh-cut fruit and vegetables [J]. Food Qual Prefer, 2017, 59: 87–96.
- [3] CHENG S. Mechanism and regulation of enzymatic browning of fresh cutfruits and vegetables [D]. Dalian: Dalian University of Technology, 2010.
- [4] MA JJ, LI FQ, HUANG MY, et al. Research progress of food-borne pathogens contamination in fresh-cut fruits and vegetables [J]. J Food SafQual, 2021, 12(7): 2591-2599.
- [5] Xue SiYue. Application Progress of Biological Preservation Technology in Fruit and Vegetable Preservation from the Perspective of Food Safety [J]. Modern Food, 2021, (15): 37-41-45.
- [6] Wang Wen, Xiao YingPing et al., Investigation of microbial contamination in *Myrica rubra* in Zhejiang Province and standard analysis [J]. Journal of Zhejiang Agricultural Sciences, 2018, 59(7): 1261–1263.
- [7] BAI Y, LIN XH, ZHU JH, et al. Quantification of Cross Contamination of *Campylobacter jejuni* during Food Preparation in a Model Kitchen in China [J]. Journal of Food Protection, 2021, 84(5): 850-856.
- [8] DIKEOU, DAVIDJG, LEEC, et al. Appearance and overall acceptability Of fresh-cut cantaloupe pieces from whole melon treated with wet steam process [J]. LWT Food Sci Technol, 2017, 82: 235–242.
- [9] Kunming University of Science and Technology, et al. Recent advances in gas detection related to food spoilage [J]. Journal of Food Safety and Quality, 2023, v. 14(06): 31-39.
- [10] FENG K, HU W Z, JIANG A L, et al. Growth potential of *Listeria monocytogenes* and *Staphylococcus aureus* on fresh-cut tropical fruits [J]. Journal of Food Science, 2015, 80(11): 2548-2554.
- [11] Zheng Rui, Carbapenem resistant characteristics and environment impact factors of clinical isolated *Enterobacteriaceae* [D]. KunMing, China: Kunming University of Science and Technology, 2017.
- [12] ABADIAS M, USALL J, ANGUERA M, et al. Microbiological quality of fresh, minimally-processed fruit and vegetables, and sprouts from retail establishments [J]. International Journal of Food Microbiology, 2008, 123(1-2): 121-129.
- [13] CARTER MQ, XUE K, BRANDL MT, et al. Functional metagenomics of *Enterobacteriaceae* O157: H7 interactions with spinach indigenous microorganisms during biofilm formation [J]. PLoS One, 2012, 7(9): 1–10.
- [14] Karch H, Denamur E, Dobrindt U, et al. The enemy with-in us: Lessons from the 2011 European *Enterobacteriaceae* O104: H4 outbreak [J]. EMBO Molecular Medicine, 2012, 4(9): 841-848.

- [15] MAHMOUDI S, POURAKBARI B, MORADZADEH M, *et al.* Prevalence and antimicrobial susceptibility of *Salmonella* and *Shigella spp.* among children with gastroenteritis in an Iranian referral hospital [J]. *Microbial Pathogen*, 2017, 109: 45–48.
- [16] CHEN JR, YAN RX, HU YF, *et al.* Compositional shifts in the fungal diversity of garlic scapes during postharvest transportation and cold storage [J]. *LWT*, 2019, 115: 108453.
- [17] JIANG LL, GUO TD, GONG QT, *et al.* Microbial community structure analysis on different phenophases of late-maturing peach ‘Snow Giant’ [J]. *Chin Agric Sci Bull*, 2022, 38(1): 44-52.
- [18] GB/T 4789.41-2016, Food safety national standard food microbiology test *Enterobacteriaceae* test [S]. Beijing, China Standard Press. Inc, 2017.
- [19] LI XT, FANG T, *et al.* Modelling growth kinetics of *Salmonella* and background microorganisms in chicken [J]. *Food Sci*, 2019, 40(9): 7-15.
- [20] CHANG Siang; LIU Yi; SHAO, *et al.* Goodness-of-fit Comparison of Prediction Models for Aerobic Bacterial Count and Shelf Life Prediction [J]. *Meat research*, 2019, v. 33; No. 242(04): 42-48.
- [21] ZHOU Guo CUI Yan YANG Wenge, *et al.* Impact of Controlled Freezing-point Storage on the *Portunus trituberculatus* Quality and Model Construction of Its Shelf-life [J]. *Acta Agriculturae Nucleatae Sinica (J Nucl Agric Sci)*, 2017, v. 31(04): 719-727.
- [22] JOSHI B, MOREIRA R G, OMAC B, *et al.* A process to decontaminate sliced fresh cucumber (*Cucumis sativus*) using electron beam irradiation [J]. *LWT-Food Science and Technology*, 2018, 91: 95-101.
- [23] LUO Haibo, HE Xiong, BAO Yonghua, *etal.* Affecting factors and possible mechanisms of quality deterioration in fresh-cut fruits and vegetables [J]. *Food Science*, 2012, 33(15): 324-330.
- [24] Yuan Yang. The Spoilage Microorganisms and Risks of Cold Dishes of Fruits and Vegetables [J]. *China Food Safety Magazine*, 2021, No. 324(31): 1-3.
- [25] Whitman R L, Shively D A, Pawlik H, *et al.* Occurrence of *Enterobacteriaceae* and enterococci in *Cladophora* (Chlorophyta) in nearshore water and beach sand of Lake Michigan [J]. *Appl Environ Microbiol*, 2003, 69 (8): 4714–4719.
- [26] MA Yue, HU Wenzhong, CHENG Shuang, *et al.* Effects of fresh-cut on the physio-biochemical changes of fruits and vegetables and its controlling methods [J]. *Science and Technology of Food Industry*, 2010, 31(2): 338-341.
- [27] COSTA JCCP, BOLÍVAR A, VALERO A, *et al.* Evaluation of the effect of *Lactobacillus sakei* strain L115 on *Listeria monocytogenes* at different conditions of temperature by using predictive interaction models [J]. *Food Res Int*, 2020, 131: 108928.
- [28] YUE XY, HU ZX, LIU XD, *et al.* Advances in predictive food microbiology model [J]. *J Zhengzhou Coll Anim Husb Eng*, 2014, 34(3): 14-16, 26.
- [29] ZHANG ZW, HE JJ, FAN CH, *et. al.* Microbial Diversity Analysis of Fresh-Cut Lotus Root During Cold Storage [J]. *Food Sci and Tech*, 2023, v. 48; No. 381(07): 16-21.
- [30] ZHAO XM, CUI H, HUANG ZH, *et. al.* Effects of Limonene Treatment on Storage Physiology and Quality of Fresh-Cut Cantaloupes [J]. *Food Research and Development*, 2023, v. 44; No. 458(13): 70-76.
- [31] GUO D, HAN YQ, WEI X, *et. al.* Relationship between Quality and Browning Physiological Changes of Fresh-cutting Apples during Cold Storage Period [J]. *Journal of Henan Agricultural Sciences*, 2020, v. 49; No. 547(08): 173-180.
- [32] HALAMICKOVA A, MALOTA L. Muscle thiobarbituric acid reactive substance of the Atlantic herring (*Clupea harengus*) in marinades collected in the market network [J]. *Acta Veterinaria Brno*, 2010, 79(2): 329-333.
- [33] CAPILLAS CR, MORAL A. Correlation between biochemical and sensory quality indices in hake stored in ice [J]. *Food Res Int*, 2001, 34(5): 441-447.
- [34] ZHANG ZH, LOU Y, DU SP, *et al.* Application and prospects of molecular biology techniques in predictive microbiology: A review [J]. *Food Science*, 2017, 38(9): 248-257.