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Research Article

Evaluating Freshness Loss of Green Tea with Q_{10} Method and Weibull Hazard Analysis under Accelerated Shelf Life Testing

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The important quality of green tea is freshness, but high temperature, light, oxygen, and humidity during storage may reduce the freshness of green tea. Thus, this study investigated the freshness loss of green tea (FLGT) under an accelerated shelf-life testing (ASLT) by sensory evaluation and acceptability test. The FLGTs of the samples stored at 20°C, 30°C, and 40°C, were determined as 67 days, 55 days, and 45 days, respectively by the Q_{10} method. In addition, they were further determined as 67 days, 57 days, and 42 days, respectively, by the Weibull Hazard Analysis (WHA). The sensory evaluation and acceptability test confirmed the correctness of the above results by 2-alternative choice analysis and chemical analysis. The Q_{10} method was applied to predict the FLGTs stored at 4°C and −20°C which were 93 days and 150 days, which was further verified by 2-alternative choice analysis and chemical analysis. The results show that it is possible to predict the suitable drinking period of green tea during storage by the Q_{10} method.

1. Introduction

Green tea (*Camellia sinensis* (L.) O. Ktze) is one of the most popular functional beverages in the world because of its antioxidant and anticancer activities and prevention of metabolic disease and neurodegenerative disease [1–4]. Basically, fresh green tea leaves normally were thermally treated to inactivate the endogenous enzymes and dehydration after harvest to yield dried green tea, and such treatments can preserve bioactive compounds and freshness attributes of green tea [5]. While green tea would lose its freshness by high temperature, light, oxygen, and humidity during storage, when green tea loses its freshness, it exhibits dark yellow color with an obviously stale aroma [6], which may decrease pharmacological activity [7]. Therefore, it is significantly important to establish a reliable method on evaluating the freshness loss of green tea (FLGT) during storage.

Accelerated shelf-life testing (ASLT) was conducted and temperature sensitivity (Q_{10}) was measured to investigate the freshness of food [8]. Accelerated shelf-life testing (ASLT) is a process of testing a product under accelerated conditions to predict the shelf life of a product under typical storage conditions [9]. Basically, elevated temperature condition is normally applied to the ASLT to expedite the deterioration process, whereas Q_{10} method is used to estimate the shelf life of a product through the chemical reaction rate alteration in response to every 10-degree rise in temperature [10]. Sensory evaluation plays an important role in the ASLT, and quality-based method and survival analysis are two common methods used in the sensory evaluation [9]. In the quality-based method, the quality difference will be established between the fresh product and products treated through ASLT [9]. For example, a seven-point scale judgement has been established by Hough and Freitas between the fresh and stored commercial ricotta cheese with 0 point as no difference and 6 points as the biggest difference [11].

Survival analysis in ASLT refers to a sensory judgement on the acceptance of a product by customers after accelerated treatment, and Weibull Hazard Analysis (WHA) is a normal analytical approach for the sensory judgement

[9]. It has been accepted that the quality turning point for a product after accelerated treatment will be determined when its sensory acceptance fails from 50% of the panelists [12]. For example, an investigation on the storage period of fresh-cut apples quality using WHA has indicated that the fresh-cut apples stored at 4°C could have 7.5-day shelf life [13]. It has been reported that Njangsa seed oil after a 21-week shelf-life period will not be accepted by more than 50% of panel judges in terms of its taste under WHA [14].

Few studies have been conducted to determine the FLGT with storage process to the best of our knowledge. Therefore, this paper applies ASLT to investigate the FLGT by sensory evaluation, the Q_{10} method, and WHA, for establishing a reliable approach to predict the FLGT.

2. Materials and Methods

2.1. Green Tea Samples and Chemicals. Huangshan Maofeng green tea (*Camellia sinensis* (L.) O. Ktze) is used as the sample in the present study. The green tea samples are provided from West Huangshan Tea Manufacturer (Huangshan, Anhui, China). According to the manufacturer, the fresh green tea leaves after harvest are immediately fixed using the thermal treatment to prevent green tea leaves from fermentation under the standard operating procedure of the green tea production. Afterwards, the green tea leaves are dehydrated to a moisture content below 5.73%, and then the dehydrated green tea leaves (9.0 kg) are immediately packed in 50 g/aluminum foil zip-lock bag with vacuum.

These green tea samples are randomly divided into 5 groups and stored at −20°C, 4°C, 20°C, 30°C, and 40°C. The stored green tea samples at −20°C can be considered as fresh tea, and the stored green tea samples at 20°C, 30°C, and 40°C, are prepared for the Q_{10} method and WHA in ASLT. The freshness duration of the stored green tea samples at 4°C will be evaluated and predicted.

Folin-Ciocalteu reagent was purchased from Beijing Solarbio Science & Technology Co., Ltd. (Beijing, China). (+)-Catechin (C), (-)-epicatechin (EC), (-)-epigallocatechin (EGC), (-)-gallocatechin (GC), (-)-epicatechin gallate (ECG), (-)-gallocatechin gallate (GCG), EGCG, and GA were purchased from Sigma-Aldrich (St. Louis, MO, USA). HPLC grade methanol and acetonitrile were a product of Tedia Co., Ltd. (Fairfield, OH, USA).

2.2. Establishing Nine-Point Scale by Sensory Evaluation. A sensory evaluation is carried out in a panel room with constant temperature (25°C) and humidity (75%) in the State Key Laboratory of Tea Plant Biology and Utilization, Anhui Agricultural University. Each tea sample (3.0 g) stored at 20°C, 30°C, and 40°C is extracted with 150 mL of boiling water for 4 min. The tea appearance and the color, aroma, and taste of tea infusion are compared with the fresh control tea leaves (stored at −20°C). A total of 6 profession panelists (a man and five women) are asked to describe their difference, from which the nine-point scale will be established according to a published method [11] with minor modifications. The overall tea sensory quality, including the appearances of tea leaves, the tea infusion color, aroma, and taste with weight coefficients of 30%, 20%, 30%, and 20%, respectively, has been evaluated by weighted sum method.

2.3. Evaluating the FLGT by the Q_{10} Method. Q_{10} is defined as the temperature sensitivity of a reaction, which describes the increase of reaction (or growth) rate or the decrease of shelf life for a 10°C increase in temperature, and it is usually assumed to be constant over a narrow range of temperature. It has been used to predict quality or nutrient losses for many foods [15]. Here, it is used to determine the FLGT of the green tea samples in ASLT. Q_{10} of the green tea samples can be calculated using the following equation [16]:

$$Q_{10}^{\Delta T/10} = \frac{\theta_{(T_1)}}{\theta_{(T_2)}}, \quad \Delta T = T_2 - T_1, \tag{1}$$

where T_1 and T_2 represent storage temperatures at 30°C and 40°C, respectively, and $\theta_{(T_1)}$ and $\theta_{(T_2)}$ are found to be the times when the tea samples stored at 30°C and 40°C lose their freshness according to the weighted score in the sensory evaluation, respectively.

2.4. Evaluating the FLGT by WHA. The WHA of these tea samples has been carried out according to a published method with minor modifications using the same panelists in tea sensory evaluation [17]. A total of 10 panelists, 4 males and 6 females with an age range of 20 to 40 years, have the basic knowledge on the sensory evaluation and complete a 2-hour tea sensory evaluation training program. There are two panelists in the beginning of the sensory evaluation. In the process of sensory evaluation, if all the panelists accept the sample to be fresh, just one more panelist is added in the next sensory evaluation. If there are unacceptable attitudes, the number of unacceptable panelists was also extra added except the former one panelist in the following sensory evaluation [18]. The panelists considered that all sensory attributes of the tea sample can be accepted by putting "✓" after the name of the tea sample; otherwise, they were marked with "✕," and the sensory evaluation would be conducted every five days until more than 50% of the panelists thought the tea sample was unaccepted. Let $h(x)$ denote a hazard value at time x obtained by

$$h(x) = \frac{1}{k}100, \tag{2}$$

where k is the reverse rank of failed products (rank k and $h(x)$ are given by columns 1 and 3 in Table 1). For each failure time, x_i, the cumulative hazard value is calculated by summing the hazard value and all preceding hazard values. The cumulative hazard can be obtained from the following equation:

$$h(T) = \sum_{I=i}^{I} ht_i. \tag{3}$$

It should be noted that the cumulative hazard values can be larger than 100%. The cumulative distribution function $F(t)$ of the Weibull model can be expressed as

Table 1: Weibull hazard ranking table for tea samples under the ASLT (20°C, 30°C, and 40°C).

Storage temperature (°C)	Reverse rank k	Age at termination (days)	Hazard 100\k	Cumulative hazard (%)
20	12	50	8.33	8.33
	11	50	9.09	17.42
	10	60	10.00	27.42
	9	65	11.11	38.54
	8	65	12.50	51.04
	7	65	14.29	65.32
	6	67	16.67	81.99
	5	67	20.00	101.99
	4	67	25.00	126.99
	3	67	33.33	160.32
	2	67	50.00	210.32
	1	67	100.00	310.32
30	18	40	5.56	5.56
	17	40	5.88	11.44
	16	45	6.25	17.69
	15	45	6.67	24.35
	14	50	7.14	31.50
	13	50	7.69	39.19
	12	50	8.33	47.52
	11	50	9.09	56.61
	10	50	10.00	66.61
	9	50	11.11	77.73
	8	55	12.50	90.23
	7	55	14.29	104.51
	6	55	16.67	121.18
	5	55	20.00	141.18
	4	55	25.00	166.18
	3	55	33.33	199.51
	2	55	50.00	249.51
	1	55	100.00	349.51
40	17	25	5.88	5.88
	16	25	6.25	12.13
	15	30	6.67	18.80
	14	30	7.14	25.94
	13	35	7.69	33.63
	12	35	8.33	41.97
	11	40	9.09	51.06
	10	40	10.00	61.06
	9	40	11.11	72.17
	8	45	12.50	84.67
	7	45	14.29	98.96
	6	45	16.67	115.62
	5	45	20.00	135.62
	4	45	25.00	160.62
	3	45	33.33	193.96
	2	45	50.00	243.96
	1	45	100.00	343.96

$$F(t) = 1 - \exp\left[-\left(-\frac{t}{\alpha}\right)\beta\right]. \quad (4)$$

The relationship between $H(t)$ and $F(t)$ can be expressed in the following form:

$$H(t) = -\ln[1 - F(t)] = \left(-\frac{t}{\alpha}\right)\beta. \quad (5)$$

So, we can perform data analysis based on this formula [19].

This probability corresponds to a Weibull percentile of 69.3%. The shape factor (β) is determined as 1/slope. The range of β is $2 < \beta < 4$, which leads to better estimations of shelf life. When the test is extended beyond shelf life, most of samples are judged as unacceptable shifting β outside the optimum range [18].

2.5. Verifying the FLGT by 2-Alternative Choice. The comparison between fresh green tea and stale green tea at 20°C, 30°C, and 40°C is carried out using the 2-alternative choice analysis based on a published method [20]. A total of 20 undergraduates majored in Tea Science in Anhui Agricultural University (Hefei, Anhui, China) participated in this experiment. These students were 9 males and 11 females and

their ages were between 20 and 30 years. These panelists have basic sensory evaluation knowledge and have been trained specifically on distinguishing the fresh and stale odor from green tea. It has been reported that a minimum number of correct judgements to establish significance at various probability levels for paired difference was 15 out of 20 total participants under a significance level of 0.05 [21]. During the analysis, two tea samples are randomly numbered and given to panelists. The panelists are asked to judge whether the two tea samples are both fresh. The FLGT is determined at regular storage temperature by the Q_{10} method. The green tea samples stored at −20°C and 4°C for 93 days and 150 days, respectively, have been compared in terms of the appearances of the leaves and the infusion colors, aromas, and tastes under a professional panel. These panelists will be asked to give score according to a nine-point scale.

2.6. Verifying the FLGT by Chemical Analysis. Contents of tea polyphenols (TPs) were determined by the Chinese National Standard GB/T 8313-2008. Catechins, including C, EC, EGC, GC, ECG, GCG, and EGCG, in the tea leaves were analyzed on an Agilent 1260 HPLC system. A Venusil C_{18} column (4.6 × 250 mm, 5 μm) was used to separate these compounds (Agilent Technologies, Santa Clara, CA, USA) under a 1.0 mL/min flow rate. Mobile phase consisted of (A) acetonitrile and (B) 1% acetic acid (v/v) in acetonitrile. A gradient program was as follows: 0 to 7 min, 10% to 30% A; 7 to 10 min, 30% A isocratic; 10 to 12 min, 30% to 60% A; 12 to 15 min, 60% to 10% A; and 15 to 20 min, 10% A isocratic. The column was maintained at 40°C during the gradient program. The injection volume was set at 5 μL and the wavelength of the ultraviolet detector was 278 nm. External standard, catechin, was used for the quantitation of catechins in the tea infusion, respectively. All data were repeated three times and recorded as mean ± standard deviation (SD).

2.7. Statistical Analysis. Data have been expressed as the mean ± standard deviation (SD) of triplicate tests. The plot between Weibull percentiles and storage time has been drafted using Origin 9.0 software (Origin Lab Corp., Northampton, MA, USA). Heat map was performed using TBtools software (https://github.com/CJ-Chen/TBtools). The 2-alternative choice data have been programmed into minimum numbers of correct judgements to establish significance at various probability levels according to a published method [21].

3. Results

3.1. Establishment of Nine-Point Scale. A nine-point scale can be established by sensory evaluation (Table 2), in which the factor with positive points means no/slight/some/visible difference with fresh tea, and the factor with negative points means entirely/visibly/slightly/some unacceptable as fresh tea. It is suggested that tea sample with a positive weighted score will be considered as fresh tea, whereas the tea with a negative weighted score will be regarded as nonfresh tea. The tea sample with zero weighted score means both acceptable and slightly unacceptable. The higher the number, the greater the acceptability. For example, a score of 4 points was regarded as no difference with fresh tea and a score of −4 points was entirely unacceptable as fresh tea. The overall tea sensory quality, including the appearances of tea leaves and the tea infusion color, aroma, and taste with weight coefficients of 30%, 20%, 30%, and 20%, respectively, has been evaluated by weighted sum method. The next Q_{10}, WHA, and 2-alternative choice analysis are all based on the nine-point scale.

3.2. Evaluating the FLGT by the Q_{10} Method Based on Nine-Point Scale. Table 3 lists the score of the attribute factors and the weighted score of these tea samples stored at 20°C, 30°C, and 40°C, respectively. We can observe that the weighted scores of all tea samples decrease during storage and eventually reach negative points, and the higher storage temperature, the less time to reach the negative points. Tea stored at 20°C, 30°C, and 40°C got negative point and lost its freshness at 70 days, 55 days, and 45 days, respectively, which indicates that the high storage temperature can accelerate freshness loss of green tea.

It is found that the FLGTs are 55 days and 45 days at 30°C and 40°C, respectively (Table 3), which can infer that Q_{10} of the green tea samples is 11/9 by equation in Methods. Additionally, the FLGT stored at 20°C can be further calculated using the following equation:

$$\theta_{(20^\circ\mathrm{C})} = \theta_{(30^\circ\mathrm{C})} \times Q^{(30-20)/10}. \quad (6)$$

In the above equation, $\theta_{(30^\circ\mathrm{C})}$ is 55 days and $Q^{(30-20)/10}$ is 11/9. The FLGT stored at 20°C can be obtained as 67 days according to the Q_{10} method. In our sensory evaluation result, the weighted score was 0.58 at 65 days and was −0.88 at 70 days, which means that the FLGT stored at 20°C is between 65 and 70 days. The FLGT obtained by the Q_{10} method agrees well with that of sensory evaluation at 20°C. The tea after the FLGT exhibits a yellowish leaves appearance and its infusion is obviously yellow color with strong stale aroma and nonfresh taste according to our panelists.

3.3. Evaluating the FLGT by WHA. In the WHA, the panelists will be asked to judge whether the tea sample after different storage periods will be still considered acceptable as fresh tea in terms of the overall quality of tea (Table 4). The overall quality of the tea samples was identified as "not accepted" when more than half of the panelists marked a "×." Table 1 shows Weibull hazard ranking for tea samples stored at an accelerated shelf-life condition (20°C, 30°C, and 40°C). According to the WHA, the betas (β) at three temperatures are 3.37, 3.13, and 3.42, respectively, which meet the requirements of Weibull distribution for β and indicate that Figure 1 can accurately predict the FLGT at three temperatures. Probability Weibull plot of time is shown on Figure 1. The FLGT is the time when the Weibull percentile reached 69.3% [18]. The FLGTs are 67 days, 57 days, and 42 days for the samples stored at 20°C, 30°C, and 40°C,

Table 2: Establishment of nine-point scale and sensory attributes description.

Score of difference	Difference from fresh tea	Description			
		Appearance (30%)	Infusion color (20%)	Infusion aroma (30%)	Infusion taste (20%)
4.0	No difference	Yellowish green, emerald and vivid	Green and bright	Clean and pure, refreshing	Mellow and fresh
3.0	Slight difference	Yellowish green, emerald and slightly vivid	Green and bright, slightly yellow	Clean and pure, less refreshing	Mellow and less fresh
2.0	Some difference	Yellowish green, emerald and some bright	Yellowish green and bright	Clean and pure with little refreshing	Mellow with little fresh
1.0	Visible difference	Yellowish green, emerald part turns yellowish green	Yellowish green	Clean and pure	Some thick feeling, without freshness
0.0	Both acceptable and slightly unacceptable	Yellowish green, no emerald	Yellowish green and the yellow part is light	Little clean and pure	Neutral, just tea taste
−1.0	Slightly unacceptable	Yellowish green or greenish yellow, the main color is undistinguishable	Greenish yellow and the yellow part is light	Unscented	A little stale taste
−2.0	Some unacceptable	Greenish yellow or yellow, obviously	Greenish yellow	a little dull odor and stale	Some stale taste
−3.0	Visibly unacceptable	Yellow and a little auburn or dry yellow	Yellow	Some stale odor	More stale taste
−4.0	Entirely unacceptable	Auburn yellow	Deep yellow	Stale odor	Stale taste

Table 3: Sensory attribute score and weighted score of tea samples under the ASLT (20°C, 30°C, and 40°C).

Storage temperature (°C)	Time (days)	Score				Weighted score
		Leaves appearance	Infusion color	Infusion aroma	Infusion taste	
20	0	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00
	5	3.50 ± 0.00	3.77 ± 0.73	3.50 ± 0.21	3.50 ± 0.80	3.00
	10	2.58 ± 0.65	3.50 ± 1.06	4.00 ± 1.04	3.50 ± 1.12	3.37
	15	2.30 ± 0.29	3.30 ± 0.58	2.40 ± 0.51	3.00 ± 0.00	2.67
	20	2.40 ± 0.42	2.87 ± 0.37	1.75 ± 0.21	3.50 ± 0.50	2.52
	25	1.80 ± 0.63	0.80 ± 0.11	0.30 ± 0.41	1.50 ± 0.58	1.09
	30	0.00 ± 0.58	0.00 ± 0.00	0.67 ± 0.58	1.83 ± 0.29	0.57
	35	2.20 ± 0.29	2.80 ± 0.76	2.00 ± 0.00	2.20 ± 0.29	2.26
	40	0.70 ± 0.58	0.60 ± 0.55	1.30 ± 0.45	1.05 ± 0.58	1.02
	45	−1.50 ± 0.80	−0.30 ± 0.00	1.50 ± 0.53	1.00 ± 0.20	0.14
	50	0.70 ± 0.52	−1.00 ± 1.10	0.40 ± 1.06	2.00 ± 0.63	0.53
	55	0.70 ± 1.11	0.30 ± 1.40	0.30 ± 0.19	1.60 ± 1.13	0.68
	60	0.42 ± 0.29	1.40 ± 0.20	0.00 ± 0.34	1.40 ± 0.53	0.69
	65	−0.16 ± 0.68	0.50 ± 0.22	0.75 ± 0.37	1.50 ± 1.11	0.58
	70	−1.00 ± 0.18	−1.00 ± 0.21	−0.50 ± 0.65	−1.00 ± 0.55	−0.88
30	0	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00
	5	2.00 ± 0.41	2.80 ± 0.41	2.80 ± 0.34	2.30 ± 0.52	2.46
	10	2.71 ± 0.46	2.20 ± 0.12	2.57 ± 1.04	2.68 ± 0.59	2.56
	15	2.80 ± 0.29	2.00 ± 1.00	3.00 ± 0.58	2.00 ± 1.00	2.54
	20	2.40 ± 0.55	2.50 ± 0.50	2.30 ± 0.74	2.60 ± 0.55	2.43
	25	1.50 ± 0.34	2.00 ± 0.39	0.16 ± 0.55	2.50 ± 1.20	1.40
	30	1.00 ± 0.19	0.00 ± 0.11	1.00 ± 0.49	1.00 ± 1.17	0.80
	35	1.00 ± 0.50	1.00 ± 0.35	1.00 ± 0.00	1.00 ± 0.90	1.00
	40	0.80 ± 1.00	−0.30 ± 0.08	−1.00 ± 0.29	−2.00 ± 0.30	0.10
	45	−1.30 ± 0.53	1.00 ± 0.00	0.00 ± 0.15	1.00 ± 0.00	0.01
	50	−0.50 ± 0.61	1.00 ± 0.46	0.00 ± 0.14	2.00 ± 0.16	0.25
	55	−1.30 ± 0.39	−1.60 ± 0.60	−1.00 ± 0.71	−0.67 ± 0.51	−1.14
	60	−0.71 ± 0.76	−1.00 ± 0.58	−1.67 ± 0.52	0.14 ± 0.69	−0.59
	65	−1.00 ± 0.75	−0.60 ± 0.75	−2.00 ± 0.39	0.00 ± 0.33	−0.62
	70	−1.60 ± 0.55	−2.30 ± 0.29	−2.20 ± 0.34	−0.20 ± 0.84	−1.64

Table 3: Continued.

Storage temperature (°C)	Time (days)	Score				Weighted score
		Leaves appearance	Infusion color	Infusion aroma	Infusion taste	
40	0	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00 ± 0.00	4.00
	5	2.50 ± 0.55	2.30 ± 0.52	2.75 ± 0.67	2.25 ± 0.32	2.49
	10	2.60 ± 0.76	3.25 ± 0.36	2.20 ± 0.57	1.80 ± 0.19	2.45
	15	1.00 ± 0.00	2.00 ± 0.00	1.00 ± 0.56	2.00 ± 0.00	1.40
	20	1.50 ± 0.50	1.25 ± 0.39	1.00 ± 0.45	1.60 ± 0.55	1.32
	25	0.40 ± 0.67	−0.25 ± 0.16	0.00 ± 0.00	0.00 ± 0.60	0.07
	30	0.50 ± 0.16	0.30 ± 0.25	−1.00 ± 0.29	1.00 ± 0.50	0.11
	35	0.75 ± 0.50	1.00 ± 0.58	1.30 ± 0.41	2.00 ± 0.58	1.22
	40	0.00 ± 0.29	−0.25 ± 0.23	0.00 ± 0.57	0.80 ± 0.00	0.11
	45	−2.00 ± 0.15	−0.67 ± 1.00	−1.67 ± 0.66	−0.50 ± 0.75	−1.34
	50	−1.70 ± 0.28	−2.17 ± 0.18	−1.80 ± 0.17	0.40 ± 0.75	−1.40
	55	−1.90 ± 0.19	−1.90 ± 0.90	−2.60 ± 0.60	−0.85 ± 0.35	−1.90
	60	−2.00 ± 0.58	−1.40 ± 0.31	−1.60 ± 0.83	−0.71 ± 0.65	−1.50
	65	−1.80 ± 0.17	−1.30 ± 0.32	−1.30 ± 0.39	−1.00 ± 0.27	−1.39
	70	−2.40 ± 0.30	−0.67 ± 0.45	−1.75 ± 1.14	−1.00 ± 0.34	−1.58

Data were the mean ± standard deviation of triplicate tests for leaves appearance, as well as infusion color, aroma, and taste.

Table 4: Acceptability test of tea samples under the ASLT (20°C, 30°C, and 40°C).

Storage temperature (°C)	Time (days)	Individual description										Overall description
20	5	✓	✓									Accept
	10	✓	✓	✓								Accept
	15	✓	✓	✓	✓							Accept
	20	✓	✓	✓	✓	✓						Accept
	25	✓	✓	✓	✓	✓	✓					Accept
	30	✓	✓	✓	✓	✓	✓	✓				Accept
	35	✓	✓	✓	✓	✓	✓	✓	✓			Accept
	40	✓	✓	✓	✓	✓	✓	✓	✓	✓		Accept
	45	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Accept
	50	✓	✓	✓	✓	✓	✓	✓	✓	× 11	× 12	Accept
	55	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	Accept
	60	✓	✓	✓	✓	✓	✓	✓	✓	✓	× 10	Accept
	65	✓	✓	✓	✓	✓	✓	✓	× 7	× 8	× 9	Accept
	70	✓	✓	✓	✓	× 1	× 2	× 3	× 4	× 5	× 6	Reject
30	5	✓	✓									Accept
	10	✓	✓	✓								Accept
	15	✓	✓	✓	✓							Accept
	20	✓	✓	✓	✓	✓						Accept
	25	✓	✓	✓	✓	✓	✓					Accept
	30	✓	✓	✓	✓	✓	✓	✓				Accept
	35	✓	✓	✓	✓	✓	✓	✓	✓			Accept
	40	✓	✓	✓	✓	✓	✓	✓	× 16	× 17		Accept
	45	✓	✓	✓	✓	✓	✓	✓	✓	× 14	× 15	Accept
	50	✓	✓	✓	✓	✓	× 9	× 10	× 11	× 12	× 13	Accept
	55	✓	✓	× 1	× 2	× 3	× 4	× 5	× 6	× 7	× 8	Reject

Table 4: Continued.

Storage temperature (°C)	Time (days)	Individual description										Overall description
	5	✓	✓									Accept
	10	✓	✓	✓								Accept
	15	✓	✓	✓	✓							Accept
	20	✓	✓	✓	✓	✓						Accept
	25	✓	✓	✓	✓	✕ 16	✕ 17					Accept
40	30	✓	✓	✓	✓	✓	✓	✓	✕ 14	✕ 15		Accept
	35	✓	✓	✓	✓	✓	✓	✓	✓	✕ 12	✕ 13	Accept
	40	✓	✓	✓	✓	✓	✓	✓	✕ 9	✕ 10	✕ 11	Accept
	45	✓	✓	✕ 1	✕ 2	✕ 3	✕ 4	✕ 5	✕ 6	✕ 7	✕ 8	Reject

A "✓" was checked when the panelists accepted the overall quality of the tea, whereas the tea with its overall quality not accepted by the panelists was given a "✕" in the analysis.

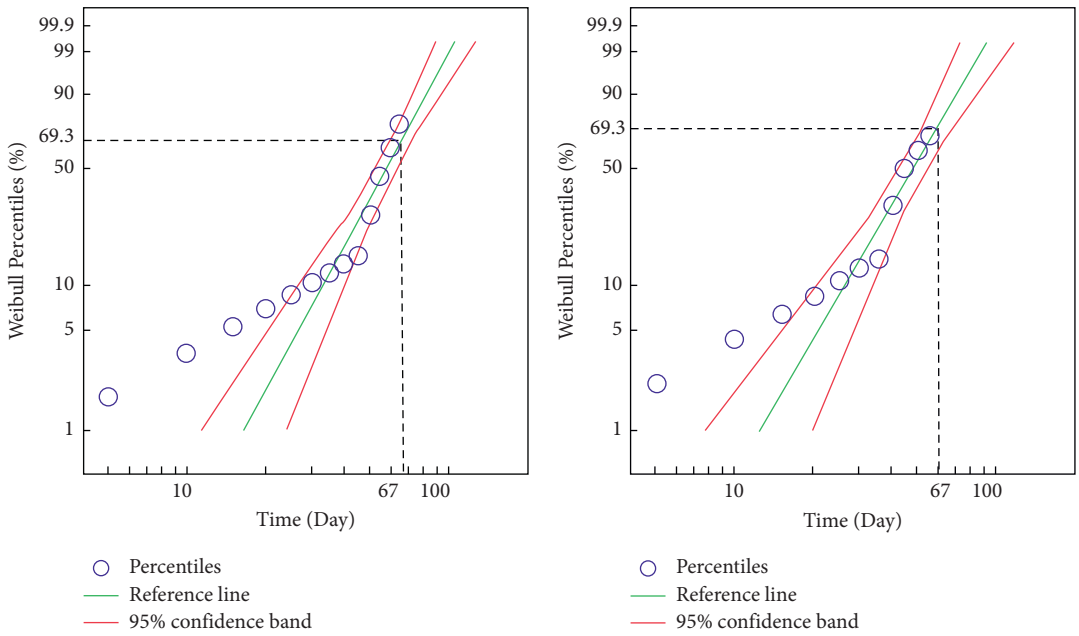
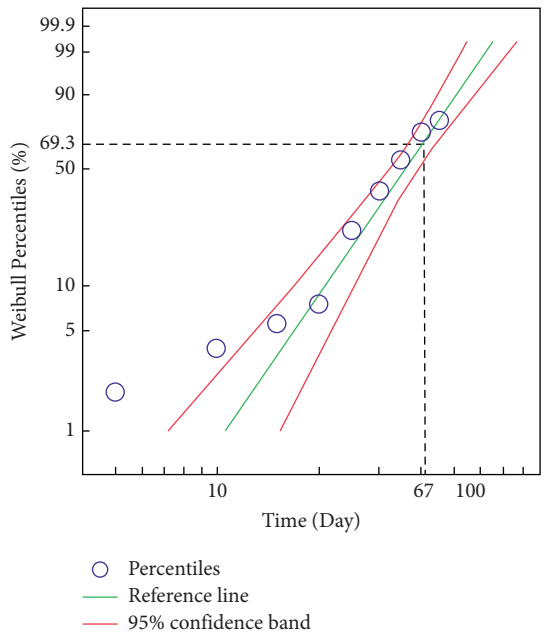


Figure 1: Continued.



(c)

Figure 1: Probability Weibull plot of time. (a) Tea samples were stored at 20°C; (b) tea samples were stored at 30°C; (c) tea samples were stored at 40°C.

respectively. Evaluations of the FLGTs by WHA are almost well consistent with the results from the sensory evaluation and the Q_{10} method.

3.4. Verifying the FLGT by 2-Alternative Choice. We find that a total of 17 panelists can distinguish the freshness difference between the fresh tea sample and the tea sample stored at 40°C for 45 days. Meanwhile, a difference on the freshness between the fresh tea sample and the tea stored at 30°C for 55 days has been also confirmed by 18 panelists. 16 panelists gave a different judgement between the fresh tea and the tea stored at 20°C for 67 days. All the three numbers are greater than 15; therefore, all these stored tea samples exhibit the significant difference compared to the fresh tea sample in terms of their freshness. The analysis of the FLGT using 2-alternative choice approach further verifies the results of the tea samples under the ASLT. This also indicates that the alterations in the quality attributes (leaves appearance, as well as tea infusion color, aroma, and taste) of the tea sample under the ASLT can provide reliable information on the investigation of freshness loss of green tea under a regular temperature condition.

3.5. Verifying the FLGT by Chemical Analysis. To further verify the correctness of the sensory results, we tested the content of tea polyphenols and catechins, including C, EC, EGC, GC, ECG, GCG, and EGCG, in the tea samples at each stage of the ASLT. The results showed that the polyphenols and catechins in the tea samples had some changes during the ASLT. Contents of GA, EGC, GCG, and ECG decreased, and contents of TPs, EC, and EGCG first increased and then decreased, while C content increased during the ASLT. Furthermore, the tea samples could be completely divided into two categories by hierarchical cluster analysis (Figure 2). The same batch of tea samples was sampled at intervals. In the early stage of storage, although the temperature was different, the compounds content in the tea samples changed little, and they were clustered into one category, which was fresh tea group (blue box in Figure 2). At the same time, the remaining samples were clustered into another category called stale tea group, in which it was worth noting that the four tea samples at 20°C for 67 days, 20°C for 65 days, 30°C for 55 days, and 40°C for 45 days were clustered together alone (red box in Figure 2), which were consistent with the results of FLGT by sensory evaluation.

3.6. Evaluating the FLGT at Regular Storage Temperature by the Q_{10} Method. According to the theory of the Q_{10} method, Q_{10} of these tea samples in this study is 11/9. Therefore, the freshness duration time for the tea sample stored at 4°C and −20°C can be theoretically predicted to be 93 days and 150 days, respectively, as shown in Table 5.

In order to verify the evaluated freshness duration for the tea samples stored at 4°C and −20°C, a sensory evaluation has been also carried out using the tea samples stored at 4°C for 93 days and the tea samples stored at −20°C for 150 days with 6 professional panelists (Table 5). Appearance of the leaves and infusion color of tea stored at 4°C for 93 days exhibit some difference/visible difference with the fresh tea. However, the aroma and taste exhibit mean with some unacceptable/slightly unacceptable appearances and get a negative overall weighted score (Table 5). The panelists describe the quality of green tea stored at 4°C for 93 days as aged and stale and the tea gets "Reject" in the acceptability

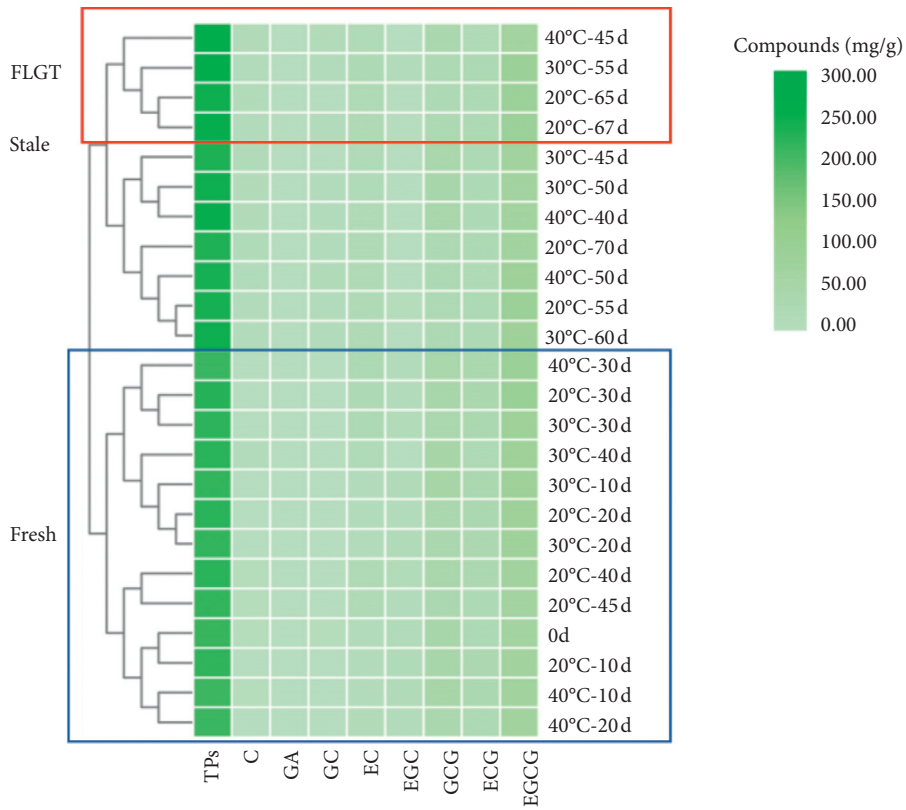


Figure 2: Hierarchical cluster analysis (HCA) for tea samples under the ASLT (20°C, 30°C, and 40°C).

Table 5: Sensory evaluation and acceptability test of FLGT stored at 4°C for 93 days and at −20°C for 150 days.

Store temperature (°C)	Time (days)	Score				Weighted score	Acceptability
		Leaves appearance	Infusion color	Infusion aroma	Infusion taste		
4	93	2.00 ± 0.35	1.00 ± 0.00	−2.00 ± 0.90	−1.00 ± 0.25	−0.18	Reject
−20	150	3.50 ± 0.45	4.00 ± 0.19	3.00 ± 0.00	2.75 ± 0.33	3.30	Accept

Data were the mean ± standard deviation of triplicate tests for leaves appearance, as well as infusion color, aroma, and taste.

test, which also verifies that the method is reliable to evaluate the FLGT by the Q_{10} method.

The appearances of the leaves and the color, aroma, and taste of the tea stored at −20°C for 150 days get positive scores, with no or slight difference compared to the fresh tea (Table 5), and the panelists describe the quality of tea as almost fresh, which means that the tea samples stored at −20°C for 150 days also remain fresh and do not reach the FLGT.

4. Conclusions

The suitable drinking period of green tea is relatively short, so it is important to explore the FLGT. In this paper, the FLGTs of tea samples stored at 20°C, 30°C, and 40°C have been evaluated to be 67 days, 55 days, and 45 days using the Q_{10} method and WHA in ASLT based on nine-point scale sensory evaluation and acceptability test session. The results have also been verified by 2-alternative choice analysis and chemical analysis. The FLGT of the tea samples stored at 4°C was predicted to be 93 days using the Q_{10} method, and the FLGT of the tea samples stored at 4°C has also been verified by acceptability test. Therefore, this paper can predict the suitable drinking period of green tea stored at a certain temperature, provide a theoretic instruction to keep fresh green tea, and suggest that researchers should conduct their experiments before the FLGT.

Abbreviations

FLGT: Freshness loss of green tea
Q_{10}: Temperature sensitivity
ASLT: Accelerated shelf-life testing
WHA: Weibull Hazard Analysis
HPLC: High-performance liquid chromatography
TPs: Tea polyphenols.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Authors' Contributions

Qianying Dai, Mengxuan Xiao, Sitong Liu, and Huozhu Jin designed the study, interpreted the results, and drafted the manuscript. Mingji Xiao, Huiqiang Wang, and Haiwei Zhang collected test data. Mengxuan Xiao and Sitong Liu contributed equally to this work.

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