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Predicting Shelf-life of Ice Cream by Accelerated Conditions

Jung-Min Park¹, Jong-Ho Koh², and Jin-Man Kim^{1,*}

¹Department of Food Marketing and Safety, Konkuk University, Seoul 05029, Korea

²Department of Bio-Food Analysis, Bio-Campus, Korea Polytechnic College, Asan 32940, Korea

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*Corresponding author : Jin-Man Kim
Department of Food Marketing and Safety,
Konkuk University, Seoul 05029, Korea
Tel: +82-2-450-3688
Fax: +82-2-455-1044
E-mail: jinmkim@konkuk.ac.kr

*ORCID
Jung-Min Park
0000-0002-8817-2856
Jong-Ho Koh
0000-0002-9727-8273
Jin-Man Kim
0000-0002-2887-8195

Abstract Shelf-life is defined as the amount of time during which a food product retains its desired sensory, chemical, and physical characteristics while remaining safe for consumption. The food industry needs to rapidly obtain the necessary information for determining the shelf life of its products. Here we studied the approaches available for conducting accelerated shelf-life tests. Accelerated shelf-life testing is applied to a variety of products to rapidly estimate change in characteristics with time. The aim of this work was to use accelerated shelf-life testing to study the changes in pH, microbiology, and sensory characteristics of ice cream by the application of a kinetic approach and, based on the observations, to estimate its shelf life. As per the current law, there is no shelf life on ice cream. Our results suggest that the shelf life of an ice cream sample was 24.27 months at -18°C , 2.29 months at -6°C , 0.39 months at -1°C , and 0.15 months at 4°C . Results of this study suggest that a set expiration date on ice cream might also contribute to effective management of ice cream characteristics in the retail chilled chain.

Keywords shelf life, ice cream, accelerated test

Introduction

Commonly loved by people of all ages, the production and consumption of ice cream continue to increase every year (Smith, 2015). Ice cream, a milk based product, is a frozen dairy dessert obtained by freezing the ice cream mix with continuous agitation and has become a dominant consumer product for majority of the population. Consumers might assume that most of the harmful microorganisms can be removed during the ice cream production process, and that it is relatively safer than other foods owing to its low temperature during storage and circulation process. As ice cream undergoes a sterilization process during manufacturing and is stored at -18°C , the manufacturer does not mark the expiration date on ice cream products in Korea; however, the manufacturer has to mark the production date as per Korean law. In addition, mentioning the expiration date for ice cream is not mandatory as per the guidelines of Codex in USA, EU, and in other countries. However, ice cream can be a

good source for microbial growth because of its nutrient content (milk protein, fat, and lactose), neutral pH, and long storage, even though it is stored in a frozen state. During the processing after the pasteurization step, there is a potential hazard of microbial growth due to addition of contaminated ingredients or improper handling of the final products including improper storage temperature. In particular, children, elderly people, and immune-suppressed patients are likely to acquire foodborne illness due to ice cream (Kanbakan et al., 2004). For example, in recent years, *Listeria monocytogenes* has been involved in numerous foodborne outbreaks that were linked to a variety of contaminated ice creams in USA (CDC, 2015) (Cartwright et al., 2013). Therefore, to establish an expiration date, characteristics indicators that can estimate the shelf-life of ice cream products are needed (Lee and Bae, 2011). In Korea, the shelf-life of a food is referred to as the “sell-by date.” When the regulations regarding labeling of information about the shelf-life are compared with those of other countries, it is compulsory for food products in Korea to display the shelf-life. However, shelf life is autonomously setup by manufacturing companies. Shelf-life is defined as the amount of time it takes for the characteristics of food to deteriorate to the point where it becomes undesirable for sale. It may also be defined as the storage time during which a food product remains safe and retains its physical, chemical, and sensory characteristics. Shelf-life dating has some benefits including several economic, environmental, and moral consequences, as it determines the maximum commercialization time for a product. For example, shelf-life dating decreases food waste at both retail and household (European Commission, 2010). Shelf-life is established typically using an accelerated shelf-life test. An accelerated shelf-life test targets food that has long-term expiration dates; it is a short-term test conducted under harsh conditions to increase the rate of chemical or physical degradation of the product. A predicted shelf-life for the specific storage environment of the product can be obtained by extrapolation. Extrapolation from exaggerated testing conditions to ambient conditions is usually done using the established relationships between kinetic parameters and the storage environment (MFDS, 2015). Generally, ice creams do not have a shelf-life. Therefore, ice cream is assessed using accelerated testing. Although there are many studies on the determination of shelf-life of milk products with short shelf-lives (Choi, 2004; Lim, 2003; Yoon et al., 2013), studies of ice cream products with a longer shelf-life are lacking. The aim of this study was to estimate the shelf-life of ice cream using proven approaches in which the ice cream was maintained at -18°C , -4°C , -1°C , and 4°C for 109 days.

Materials and Methods

Samples and experimental design

Ice creams were selected and purchased from a local market at Seoul in Korea. Ice cream (I1 and I2) manufactured by two different companies were selected. Ice cream (I1) containing has 10% of milk fat and not less than 16% of milk solids, and ice cream (I2) has containing 14% of milk fat and not less than 16% of milk solids. Ice cream defined that samples is containing not less than 6% of milk fat and not less than 16% of milk solids (Ministry of Food and Drug Safety, MFDS, 2017). Therefore, two samples type of products is ice cream.

Microbiological analysis

For all microbiological experiments, the samples were serially diluted in sterile distilled water, and all experiments were performed in triplicate. The data was reported as the mean of three independent experiments. Microbiological analyses were performed according to guidelines of the Ministry of Food and Drug Safety (MFDS, 2015). Aerobic plate counts were measured using plate count agar (Difco, Detroit, USA) and incubated at 35°C for 48 h. For coliform counts, the samples

were plated on the surface of deoxycholate agar (Difco) and incubated at 37°C for 48 h. *Listeria monocytogenes* growth was assessed by surface plating on PALCAM (Polymyxin Acriflavin LiCl Ceftazidime Esculin Mannitol) agar (Difco) followed by incubation at 35°C for 48 h. *Staphylococcus aureus* growth was determined by surface plating on Mannitol Salt-Egg Yolk agar (Difco) followed by incubation at 35°C for 48 h. All analyses were performed in triplicate. All microbiological analyses were performed according to the *Process Criteria and Ingredient Standard of Livestock Products* published by MFDS.

pH and sensory evaluation

The pH of homogenized ice cream was determined using a digital pH meter (Model AM-7, Nissei, Japan). Sensory evaluation of the ice cream samples was conducted by ten panelists. Visual acceptability, texture (in mouth, expressed as juiciness), color, aroma flavor or taste and overall impression were rated based on the preference using a 9-point hedonic scale (1: extremely dislike to 9: extremely like). Off-odor analysis was carried out using a nine point scale where 1: absent or poor characteristic to 9: too intense or excellent. Panelists were motivated to express criticisms on the score sheets used for the sensory evaluation (Lebesi and Tzia, 2011; Meilgaard et al., 1991). All analyses were performed in triplicate.

Estimation of shelf-life by accelerated tests

In general, actual testing is the primary method used to determine shelf life. However, if the shelf-life of a product is more than three months, shelf-life tests can take a long time and would be expensive. Therefore, accelerated shelf-life testing is applied to a variety of products to estimate change in characteristics over a short period of time. Accelerated shelf-life testing was used to study the changes in pH, microbiology, and sensory characteristics of ice cream by the application of a kinetic approach and, based on the observations, its shelf life was estimated. Accelerated shelf-life tests allow estimation of shelf lives in a short period of time using experimental conditions that are harsher than the actual distribution conditions. Results obtained from these experiments are used to estimate shelf-life using the Arrhenius equation. Arrhenius equation was used to choose the sell-by date. The Arrhenius equation is as follows:

$$\ln K = -(E_a/R) \times 1/T + \ln A$$

This equation was used for each indicator. In the equation, k is the reaction rate constant, A is the constant, E_a is the activation energy (kcal/mol), R is the universal gas constant ($R=1.987 \text{ cal} \cdot \text{mol}^{-1} \cdot \text{K}^{-1}$), and T is the absolute temperature (K). (Haralampu et al., 1985; Singh, 1994)

Statistical analysis

Statistical analysis was carried out on all variables, and was measured using the General Linear Model (GLM) procedure in SAS software (SAS, 2011). A software program using Duncan's multiple range test to compare treatment means was applied. A value of $p < 0.05$ was considered statistically significant. All data were expressed as mean \pm SD.

Results and Discussion

Microbiological analysis

Changes in the microbial growth of ice cream during storage periods at -18°C , -6°C , -1°C , and 4°C for 109 days are

presented in Table 1. During this time, the characteristics changed with microbiological, pH, and sensory characteristics. The initial aerobic plate count of sample I1 was 1.00 ± 0.02 Log CFU/mL. After 109 days, values were 1.47 ± 0.12 Log CFU/mL, 2.66 ± 0.01 Log CFU/mL, and 4.02 ± 0.05 Log CFU/mL at -18°C , -6°C , and -1°C , respectively; after 53 days, the value was 4.49 ± 0.02 Log CFU/mL at 4°C . The initial value for sample I2 was 1.20 ± 0.05 Log CFU/mL. After 109 days, the aerobic plate count values were 1.34 ± 0.06 Log CFU/mL, 2.81 ± 0.07 Log CFU/mL, and 3.88 ± 0.01 Log CFU/mL at -18°C , -6°C , and -1°C , respectively; after 53 days, the value was 2.72 ± 0.04 Log CFU/mL at 4°C ($p < 0.05$) (Table 1). Consistent with these observations, Kim et al. (2005) reported that total aerobic count of a commercially available ice cream was detected to be 1–2 Log CFU/g in Korea. In this study, coliform, *Listeria monocytogenes*, and *Staphylococcus aureus* were not detected at any storage temperature during whole period. The legal characteristics limit was defined according to the *Process Criteria and Ingredient Standard of Livestock Products* described by the MFDS (2015). The maximum acceptable levels for total aerobic counts is $n=5$, $c=2$, $m=10,000$, and $M=100,000$; for coliform counts is less than $n=5$, $c=2$, and $m=100$; and for *Listeria monocytogenes* is less than $n=5$, $c=2$, $m=0$, and $M=0/25$ g. The non-legal characteristics limit was calculated based on a regression equation using Y values for each characteristic indicator, and X values for each sensory evaluation; a value of 5 points (characteristics limit of sensory evaluation using a nine-point hedonic scale) was substituted for sensory ability. The shelf-life was then predicted based on a regression equation using Y values for the indicator, and X values for the storage period after substituting the characteristics limit. The shelf-life was estimated as the shortest time to reach the characteristics limit as assessed by characteristics indicators.

Table 1. Changes in the total aerobic counts of ice cream

Storage period (d)	I1				I2			
	-18°C	-6°C	-1°C	4°C	-18°C	-6°C	-1°C	4°C
0	1.00 ± 0.02^f	1.00 ± 0.02^j	1.00 ± 0.02^n	1.00 ± 0.02^g	1.20 ± 0.05^b	1.20 ± 0.05^f	1.20 ± 0.05^i	1.20 ± 0.05^s
5	1.24 ± 0.01^e	1.40 ± 0.13^i	1.00 ± 0.09^n	1.25 ± 0.02^f	1.18 ± 0.03^b	1.30 ± 0.01^f	1.18 ± 0.05^i	1.61 ± 0.11^f
11	1.24 ± 0.04^e	1.48 ± 0.05^i	1.18 ± 0.02^m	1.32 ± 0.11^f	1.30 ± 0.06^{ab}	1.30 ± 0.16^f	2.08 ± 0.02^h	1.70 ± 0.05^f
18	1.26 ± 0.06^e	1.54 ± 0.07^{hi}	1.18 ± 0.05^m	1.68 ± 0.05^e	1.33 ± 0.14^a	1.48 ± 0.05^e	2.19 ± 0.00^g	1.91 ± 0.00^e
25	1.28 ± 0.04^{de}	1.60 ± 0.05^h	1.41 ± 0.02^l	1.74 ± 0.02^e	1.33 ± 0.16^a	1.48 ± 0.11^d	2.20 ± 0.04^g	2.03 ± 0.05^{de}
32	1.31 ± 0.14^{cde}	1.90 ± 0.12^h	1.70 ± 0.06^k	2.66 ± 0.13^d	1.34 ± 0.02^a	1.65 ± 0.02^c	2.32 ± 0.14^f	2.12 ± 0.12^{cd}
39	1.33 ± 0.03^{bcde}	1.98 ± 0.07^g	2.23 ± 0.03^j	3.63 ± 0.05^c	1.34 ± 0.02^a	1.95 ± 0.07^b	2.51 ± 0.01^e	2.24 ± 0.13^{bc}
46	1.35 ± 0.03^{abcde}	2.02 ± 0.01^{fg}	2.37 ± 0.02^i	4.09 ± 0.06^b	1.34 ± 0.05^a	2.34 ± 0.02^b	2.79 ± 0.00^d	2.33 ± 0.04^b
53	1.37 ± 0.06^{abcde}	2.06 ± 0.03^{efg}	2.63 ± 0.01^h	4.49 ± 0.02^a	1.34 ± 0.12^a	2.34 ± 0.05^a	2.81 ± 0.11^d	2.72 ± 0.04^a
63	1.39 ± 0.14^{abcde}	2.13 ± 0.02^{def}	2.80 ± 0.07^g		1.34 ± 0.03^a	2.77 ± 0.08^a	2.81 ± 0.05^d	
67	1.41 ± 0.13^{abcd}	2.15 ± 0.05^d	3.06 ± 0.12^f		1.34 ± 0.00^a	2.77 ± 0.03^a	2.85 ± 0.05^d	
74	1.42 ± 0.06^{abc}	2.17 ± 0.03^d	3.43 ± 0.03^e		1.34 ± 0.01^a	2.77 ± 0.01^a	2.94 ± 0.01^c	
81	1.43 ± 0.03^{abc}	2.32 ± 0.07^d	3.63 ± 0.13^d		1.34 ± 0.01^a	2.78 ± 0.0^a	2.96 ± 0.03^c	
88	1.44 ± 0.02^{abc}	2.32 ± 0.14^d	3.63 ± 0.0^d		1.35 ± 0.03^a	2.78 ± 0.03^a	2.97 ± 0.05^c	
95	1.45 ± 0.02^{abc}	2.34 ± 0.07^{bc}	3.73 ± 0.02^c		1.35 ± 0.11^a	2.78 ± 0.13^a	3.70 ± 0.04^b	
102	1.46 ± 0.05^{ab}	2.45 ± 0.09^b	3.83 ± 0.01^b		1.35 ± 0.04^a	2.80 ± 0.05^a	3.79 ± 0.07^{ab}	
109	1.47 ± 0.12^a	2.66 ± 0.01^a	4.02 ± 0.05^a		1.34 ± 0.06^a	2.81 ± 0.07^a	3.88 ± 0.01^a	

All values are mean standard deviation of three replicates.

^{a-n} Means within a column with different letters are significantly different ($p < 0.05$).

pH

The pH values of ice cream samples during storage are shown in Table 2 and 3. The initial pH of I1 and I2 were 6.42 ± 0.03 and 6.42 ± 0.06 , respectively. After storage for 109 days, the pH of I1 was 6.55 ± 0.04 , 6.65 ± 0.05 , and 6.53 ± 0.04 at -18°C , -6°C , and -1°C , respectively; and it was 6.88 ± 0.03 after incubation for 53 days at 4°C . The pH of I2 was 6.65 ± 0.05 , 6.65 ± 0.05 , and 6.65 ± 0.15 , at -18°C , -6°C , and -1°C , respectively; and 6.77 ± 0.06 after incubation for 53 days at 4°C ($p < 0.05$).

Sensory evaluation

Sensory shelf-life studies usually involve measuring the intensity of different sensory characteristics throughout storage period, until they reach a failure criteria or cut-off point, which corresponds to the maximum tolerable deterioration (Gimenez et al., 2012). Numerous studies have used sensory evaluation as an indicator of food characteristics (Lu et al., 2011; Park, 2013; Peryam and Pilgrim, 1957). Changes in the sensory characteristics of ice cream are presented in Table 2 and 3. Sensory evaluation was performed using a 9-point hedonic scale (MFDS, 2015); the evaluation criteria were defined such that the initial characteristics level was 9 points, and the characteristics limit was 5 points. Sensory characteristics decreased significantly during the experimental period ($p < 0.05$), and decreased more rapidly at higher storage temperatures (Derossi et al., 2016). The sensory evaluation of I1 and I2 began to change after 5 days at -6°C , -1°C , and 4°C , but were unchanged on storage at -18°C . The sensory characteristics of I1 remained above 5 points when stored at -18°C and -6°C , and for 32 days and 18 days when stored at -1°C and 4°C , respectively. The sensory characteristics of I2 remained greater than 5 points when

Table 2. The pH and sensory of ice cream (I1) during storage at -18°C , -6°C , -1°C , and 4°C

Day/ -18°C	pH	Sensory	Day/ -6°C	pH	Sensory	Day/ -1°C	pH	Sensory	Day/ 4°C	pH	Sensory
0	6.42 ± 0.03^e	9.00 ± 0.00^a	0	6.42 ± 0.03^f	9.00 ± 0.00^a	0	6.42 ± 0.03^b	9.00 ± 0.00^a	0	6.42 ± 0.03^d	9.00 ± 0.00^a
5	6.44 ± 0.06^{de}	9.00 ± 0.00^a	5	6.46 ± 0.05^{def}	7.67 ± 1.15^{ab}	5	6.45 ± 0.03^{ab}	7.76 ± 1.15^b	5	6.63 ± 0.02^c	8.00 ± 0.00^b
11	6.44 ± 0.03^{de}	9.00 ± 0.00^a	11	6.46 ± 0.00^{cdef}	7.67 ± 1.15^{ab}	11	6.47 ± 0.04^{ab}	7.33 ± 1.53^b	11	6.74 ± 0.05^b	6.67 ± 1.15^c
18	6.44 ± 0.06^{de}	9.00 ± 0.00^a	18	6.44 ± 0.06^{ef}	7.67 ± 1.15^{ab}	18	6.47 ± 0.09^{ab}	6.67 ± 0.58^{bc}	18	6.60 ± 0.02^c	5.33 ± 0.58^d
25	6.48 ± 0.04^{bcde}	9.00 ± 0.00^a	25	6.52 ± 0.00^{abcde}	7.67 ± 1.15^{ab}	25	6.49 ± 0.03^{ab}	6.00 ± 0.00^{cd}	25	6.80 ± 0.05^{ab}	4.33 ± 0.58^e
32	6.49 ± 0.02^{bcde}	9.00 ± 0.00^a	32	6.53 ± 0.04^{abcde}	7.67 ± 1.15^{ab}	32	6.50 ± 0.09^{ab}	5.33 ± 1.15^{de}	32	6.83 ± 0.03^{ab}	3.33 ± 0.58^f
39	6.49 ± 0.00^{bcde}	9.00 ± 0.00^a	39	6.50 ± 0.21^{bcde}	7.67 ± 1.15^{ab}	39	6.51 ± 0.02^{ab}	4.67 ± 0.58^{ef}	39	6.87 ± 0.07^a	1.67 ± 0.58^g
46	6.48 ± 0.00^{bcde}	9.00 ± 0.00^a	46	6.51 ± 0.07^{abcde}	7.67 ± 1.15^{ab}	46	6.52 ± 0.00^{ab}	4.00 ± 1.00^{fg}	46	6.87 ± 0.03^a	1.33 ± 0.58^g
53	6.52 ± 0.07^{bc}	9.00 ± 0.00^a	53	6.56 ± 0.05^{abcde}	7.33 ± 1.53^b	53	6.51 ± 0.05^{ab}	3.33 ± 0.58^{gh}	53	6.88 ± 0.03^a	1.00 ± 0.00^g
63	6.50 ± 0.01^{bcd}	9.00 ± 0.00^a	63	6.55 ± 0.01^{abcde}	7.00 ± 0.00^b	57	6.55 ± 0.01^a	2.67 ± 1.15^h			
67	6.51 ± 0.07^{bcd}	9.00 ± 0.00^a	67	6.57 ± 0.13^{abcde}	7.00 ± 0.00^b	64	6.51 ± 0.02^{ab}	2.33 ± 0.58^{hi}			
74	6.52 ± 0.02^b	9.00 ± 0.00^a	74	6.58 ± 0.00^{abcd}	7.00 ± 0.00^b	71	6.51 ± 0.04^{ab}	1.00 ± 0.00^i			
81	6.52 ± 0.03^b	9.00 ± 0.00^a	81	6.59 ± 0.06^{abc}	7.00 ± 0.00^b	78	6.52 ± 0.03^{ab}	1.00 ± 0.00^i			
88	6.53 ± 0.05^{ab}	9.00 ± 0.00^a	88	6.61 ± 0.02^{ab}	7.00 ± 0.00^b	85	6.52 ± 0.11^{ab}	1.00 ± 0.00^i			
95	6.53 ± 0.02^{ab}	9.00 ± 0.00^a	95	6.62 ± 0.03^{ab}	7.00 ± 0.00^b	92	6.53 ± 0.10^{ab}	1.00 ± 0.00^i			
102	6.60 ± 0.03^a	9.00 ± 0.00^a	102	6.62 ± 0.01^{ab}	7.00 ± 0.00^b	99	6.56 ± 0.12^a	1.00 ± 0.00^i			
109	6.55 ± 0.04^{ab}	9.00 ± 0.00^a	109	6.65 ± 0.05^a	7.00 ± 0.00^b	106	6.53 ± 0.04^{ab}	1.00 ± 0.00^i			

All values are mean standard deviation of three replicates.

^{a-g} Means within a column with different letters are significantly different ($p < 0.05$).

Table 3. The pH and sensory of ice cream (I2) during storage at -18°C , -6°C , -1°C , and 4°C

Day/ -18°C	pH	Sensory	Day/ -6°C	pH	Sensory	Day/ -1°C	pH	Sensory	Day/ 4°C	pH	Sensory
0	6.42±0.06 ^c	9.00±0.00 ^a	0	6.42±0.06 ^g	9.00±1.00 ^a	0	6.42±0.06 ^e	9.00±0.00 ^a	0	6.42±0.06 ^e	9.00±0.00 ^a
5	6.39±0.07 ^e	9.00±0.00 ^a	5	6.64±0.08 ^f	7.67±0.58 ^b	5	6.45±0.04 ^{bc}	7.76±0.58 ^b	5	6.55±0.05 ^b	7.67±1.15 ^b
11	6.40±0.02 ^e	9.00±0.00 ^a	11	6.62±0.03 ^f	7.33±0.58 ^b	11	6.47±0.06 ^{bc}	6.67±1.15 ^{bc}	11	6.60±0.02 ^b	6.67±1.15 ^{bc}
18	6.41±0.03 ^e	9.00±0.00 ^a	18	6.66±0.10 ^{def}	7.00±0.00 ^{bc}	18	6.46±0.06 ^{bc}	6.33±0.58 ^{cd}	18	6.66±0.06 ^{ab}	6.33±0.58 ^{cd}
25	6.47±0.03 ^{de}	9.00±0.00 ^a	25	6.61±0.04 ^f	7.00±1.00 ^{bc}	25	6.47±0.01 ^{bc}	6.00±0.00 ^{cde}	25	6.72±0.15 ^a	5.33±0.58 ^{de}
32	6.58±0.10 ^{bcd}	9.00±0.00 ^a	32	6.64±0.06 ^{ef}	6.67±0.58 ^{bcd}	32	6.50±0.02 ^{abc}	5.33±1.15 ^{def}	32	6.75±0.06 ^a	5.33±0.5 ^{de}
39	6.50±0.08 ^{cde}	9.00±0.00 ^a	39	6.61±0.07 ^e	6.67±0.58 ^{bcd}	39	6.51±0.09 ^{abc}	5.00±1.00 ^{ef}	39	6.78±0.07 ^a	5.00±0.00 ^{ef}
46	6.63±0.11 ^{abc}	9.00±0.00 ^a	46	6.59±0.06 ^e	6.67±1.53 ^{bcd}	46	6.52±0.07 ^{abc}	5.00±1.00 ^{ef}	46	6.74±0.07 ^a	4.33±0.58 ^{ef}
53	6.66±0.08 ^{ab}	9.00±0.00 ^a	53	6.80±0.05 ^{abc}	5.67±0.58 ^{cde}	53	6.54±0.04 ^{abc}	5.00±1.00 ^{ef}	53	6.77±0.06 ^a	4.00±0.00 ^f
63	6.61±0.10 ^{abc}	9.00±0.00 ^a	63	6.74±0.06 ^{cde}	5.67±0.58 ^{cde}	57	6.53±0.04 ^{abc}	5.00±0.00 ^{ef}			
67	6.64±0.06 ^{ab}	9.00±0.00 ^a	67	6.76±0.06 ^{def}	5.33±0.58 ^{de}	64	6.54±0.04 ^{abc}	4.67±0.58 ^{ef}			
74	6.66±0.04 ^{ab}	9.00±0.00 ^a	74	6.79±0.06 ^{abc}	5.33±0.58 ^{de}	71	6.58±0.09 ^{ab}	4.67±0.58 ^{ef}			
81	6.69±0.07 ^{ab}	9.00±0.00 ^a	81	6.82±0.04 ^{abc}	5.67±0.58 ^{cde}	78	6.57±0.06 ^{abc}	4.33±0.58 ^f			
88	6.71±0.11 ^{ab}	9.00±0.00 ^a	88	6.84±0.08 ^{abc}	5.67±0.58 ^{cde}	85	6.58±0.13 ^{ab}	4.67±0.58 ^{ef}			
95	6.74±0.08 ^a	9.00±0.00 ^a	95	6.87±0.07 ^{ab}	5.33±0.58 ^{de}	92	6.59±0.11 ^b	4.33±0.58 ^f			
102	6.69±0.11 ^{ab}	9.00±0.00 ^a	102	6.89±0.05 ^a	5.33±0.58 ^{de}	99	6.58±0.12 ^{ab}	4.33±0.58 ^f			
109	6.65±0.05 ^{ab}	9.00±0.00 ^a	109	6.65±0.05 ^{ef}	5.00±1.00 ^e	106	6.65±0.15 ^a	4.00±0.00 ^f			

All values are mean standard deviation of three replicates.

^{a-g} Means within a column with different letters are significantly different ($p < 0.05$).

stored at -18°C and -6°C , and for 57 days and 39 days when stored at -1°C and 4°C , respectively.

Estimation of the shelf-life of ice cream by accelerated tests

Accelerated shelf-life testing is used a variety of products with long expiration period to rapidly estimate changes in characteristics. (Breda et al., 2012; Chandler and McMeekin, 1989; Lee et al., 2006; Taoukis et al., 1999). Initially, the changes in characteristics displayed first-order kinetics. Instead of a separate estimate of the reaction rate (k) at different temperatures followed by use of Arrhenius equation to estimate the other parameters, a one-step multiple linear regression, which fit data versus time for all tested temperatures, was followed. The influence of temperature on the reaction rate was described using the modified Arrhenius equation. Regression equation, and value of r^2 date for ice cream at -18°C , -6°C , -1°C , and 4°C are shown in Table 4. The date of attaining characteristics limit for ice cream sample I1, with respect to each characteristics criteria -18°C , -16°C , -1°C , and 4°C was as follows: total aerobic counts, 433.61 months, 15.51 months, 4.19 months, and 1.18 months; pH, 24.27 months, 4.17 months, 0.98 months, and 1.88 months; sensory evaluation, 626.68 months, 5.51 months, 0.87 months, and 0.15 months, respectively. The shelf-life was defined as the earliest date among all the dates of attaining characteristics limit when each characteristics criteria reached to its limit. Shelf-life of ice cream I1 was estimated to be 24.27 months at -18°C , 4.17 months at -6°C , 0.87 months at -1°C , and 0.15 months at 4°C . The date of attaining characteristics limit for ice cream sample I2, with respect to each characteristics criteria at -18°C , -6°C , -1°C , and 4°C was follows: total aerobic counts, 27.98 months, 5.09 months, 2.62 months, and 1.38 months; pH, 36.32 months, 2.29 months, 0.39 months, and 0.44 months; sensory

Table 4. Evaluation of the linear regression equation for the estimated shelf-life of ice cream

Characteristics indicator	Temperature (°C)	Zero-reaction		First-reaction		
		Regression equation	r ²	Regression equation	r ²	
I1 Total aerobic counts (CFU/mL) pH Sensory	-18 -6 -1 4	y=0.0014x+1.2651	0.7217	y=0.0016x+0.1996	0.7751	
		y=0.0087x+1.2634	0.9663	y=0.0053x+0.3647	0.9486	
		y=0.0310x+0.8711	0.9767	y=0.0140x+0.0678	0.9376	
		y=0.1140x+0.8080	0.9142	y=0.0486x+0.0589	0.9459	
	-18 -6 -1 4	y=0.0009x+6.4482	0.9219	y=0.0001x+1.8639	0.9210	
		y=0.0007x+6.4913	0.6545	y=0.0001x+1.8704	0.6537	
		y=0.0009x+6.4582	0.7318	y=0.0001x+1.8653	0.7312	
		y=0.0149x+6.5657	0.7246	y=0.0022x+1.8818	0.7181	
	-18 -6 -1 4	y=-0.0028x+9.1830	0.3211	y=-0.0003x+2.2188	0.3211	
		y=-0.0098x+7.8756	0.4839	y=-0.0014x+2.0686	0.9005	
		y=-0.0971x+8.4915	0.9909	y=-0.0239x+2.3195	0.8909	
		y=-0.3579x+8.4732	0.9900	y=-0.0929x+2.3034	0.9556	
	I2 Total aerobic counts (CFU/mL) pH Sensory	-18 -6 -1 4	y=0.0070x+1.5260	0.9600	y=0.0012x+0.2107	0.8177
			y=0.0126x+1.7711	0.6836	y=0.0055x+0.4352	0.8884
			y=0.0139x+1.6920	0.7952	y=0.0087x+0.4680	0.8074
			y=0.0359x+1.6496	0.9288	y=0.0173x+0.5123	0.9436
-18 -6 -1 4		y=0.0018x+6.4743	0.8199	y=0.0003x+1.8679	0.8157	
		y=0.0015x+6.6080	0.7056	y=0.0002x+1.8883	0.7015	
		y=0.0017x+6.4375	0.9511	y=0.0003x+1.8622	0.9514	
		y=0.0128x+6.4941	0.7951	y=0.0019x+1.8709	0.7930	
-18 -6 -1 4		y=-0.0028x+9.1830	0.3211	y=-0.0003x+2.2188	0.3211	
		y=-0.0253x+7.6734	0.9582	y=-0.0056x+2.1309	0.9501	
		y=-0.0362x+7.3733	0.8432	y=-0.0065x+2.0121	0.9061	
		y=-0.3613x+7.9904	0.9577	y=-0.1038x+2.2433	0.9635	

evaluation, 72.86 months, 2.72 months, 0.75 months, and 0.22 months, respectively. The shelf-life was defined as the earliest date among all dates of attaining characteristics limit when each characteristics criteria reached to its limit. Shelf-life of ice cream sample I2 was estimated to be 27.98 months at -18°C, 2.29 months at -6°C, 0.39 months at -1°C, and 0.22 months at 4°C (Table 5). Finally, summarizing the results, Table 6 shows the shelf-life of ice cream was estimated after 24.27 months at -18°C, 2.29 months at -6°C, 0.39 months at -1°C, and 0.15 months at 4°C. However, the shelf-lives estimated in this study cannot be applied to all ice creams, because many factors, including the manufacturing process and packing materials, also play vital roles. All food expiration dates could be established as self-applied safety factor by each company. In addition, to determine the shelf-life, other factors such as storage conditions, temperature of storage, etc. should be considered. For ice cream, the expiration date is not mandatory because ice cream is circulated in the frozen state at -18°C or less, with minimal risk of deterioration. But, the consumer insists on an expiration date owing to consumer's own safety and right to know (Da Silva et al., 2014; Jeon et al., 2013). However, all ice cream samples could not be applied, therefore, this study aims to obtain basic information to establish shelf-life.

Table 5. Regression equations of ice cream stored at -18°C , -6°C , -1°C , and 4°C

Characteristics indicator	Temperature (T)	Reaction order	Regression equation	Ea (kal/mol) ¹⁾	K ²⁾	A ₀ ³⁾ -A _t ⁴⁾	Shelf-life (month)	
I1 Total aerobic counts (CFU/mL)	255	Zero	LnK=-19025x+66.498	37.80	0.0003	4.00	433.61	
	267				0.0086	4.00	15.51	
	272				0.0319	4.00	4.19	
	277				0.1126	4.00	1.18	
	pH	255	Zero	LnK=-6566.8x+18.456	13.05	0.0007	0.49	24.27
		267				0.0022	0.27	4.17
		272				0.0034	0.10	0.98
		277				0.0052	0.30	1.88
	Sensory	255	Zero	LnK=-26860x+96.878	53.37	0.0002	4.00	626.68
		267				0.0242	4.00	5.51
		272				0.1538	4.00	0.87
		277				0.9144	4.00	0.15
I2 Total aerobic counts (CFU/mL)	255	Zero	LnK=-9664.8x+32.428	19.20	0.0042	3.52	27.98	
	267				0.0231	3.52	5.09	
	272				0.0449	3.52	2.62	
	277				0.0852	3.52	1.38	
	pH	255	First	LnK=-10770x+33.175	21.40	-9.0603	0.13	36.32
		267				-7.1621	0.05	2.29
		272				-6.4206	0.02	0.39
		277				-5.7059	0.04	0.44
	Sensory	255	First	LnK=-18664x+64.971	37.09	-8.2212	0.59	72.86
		267				-4.9316	0.59	2.72
		272				-3.6467	0.59	0.75
		277				-2.4081	0.59	0.22

¹⁾ Activation energy in kJ/mol.

²⁾ Rate constant.

³⁾ Initial data of index characteristics attribute.

⁴⁾ Data of index characteristics attribute as t time passes.

Table 6. Estimated shelf-life of ice cream at -18°C , -6°C , -1°C , and 4°C

Temperature ($^{\circ}\text{C}$)	Shelf life (mon)
-18	24.27
-6	2.29
-1	0.39
4	0.15

Conflicts of Interest

The authors declare no potential conflict of interest.

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