

Application of a Prototype of Microbial Time Temperature Indicator (TTI) to the Prediction of Ground Beef Qualities during Storage

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Abstract

The predictive ability for off-flavor development and quality change of ground beef was evaluated using a microbial time temperature indicator (TTI). Quality indices such as off-flavor detection (OFD) time, color, pH, volatile basic nitrogen (VBN), aerobic mesophilic bacteria (AMB) counts, and lactic acid bacteria (LAB) counts were measured during storage at 5, 10, 15, and 25°C, respectively. Arrhenius activation energies (E_a) were estimated for temperature dependence. The E_a values for TTI response (changes in titratable acidity (TA)), VBN, AMB counts, LAB counts, and freshness, which is defined based on OFD time for quality indices of ground beef, were 106.22 kJ/mol, 58.98 kJ/mol, 110.35 kJ/mol, 116.65 kJ/mol, and 92.73 kJ/mol, respectively. The E_a of microbial TTI was found to be closer to those of the AMB counts, LAB counts, and freshness. Therefore, AMB counts, LAB counts, and freshness could be predicted accurately by the microbial TTI response due to their E_a similarity. The microbial TTI exhibited consistent relationships between its TA change and corresponding quality indices, such as AMB counts, LAB counts, and freshness, regardless of storage temperature. Conclusively, the results established that the developed microbial TTI can be used in intelligent packaging technology for representing some selected quality indices of ground beef.

Key words: ground beef, time temperature indicator (TTI), quality changes, kinetics, temperature dependence

Introduction

As the income of today's family has increased, so have the number and frequency of meat consumption. A recent report showed that consumption of beef in South Korea grew more than 31% in the past four years. South Koreans consumed an average of 8.8 kg for beef per person in 2010 (KMTA, 2011). Increased beef consumption of South Koreans has brought higher consumer concerns about beef safety.

Ground beef is considered by many to be the most popular and most versatile of all bovine products (Brewer, 2012). Ground beef is more perishable than whole muscle cuts of meat and should be handled with particular care. If any bacteria are present on the surface, the grinding process mixes it throughout the entire product. This process results in shorter shelf-life. Shelf-life of ground beef

also can vary widely depending upon temperature control, packaging and distribution systems. During storage and distribution, the quality of meats deteriorates in the first instance because of discoloration, secondly because of oxidative rancidity of lipids and thirdly on account of microbial changes (Pearson and Tauber, 1984). Beef quality changes are usually evaluated in terms of volatile basic nitrogen (VBN), thiobarbituric acid values (TBA), lactic acid bacteria (LAB) count, aerobic mesophilic bacteria (AMB) count, water loss, pH, surface color, and off-flavor detection (OFD) times (Guerrero and Chabela, 1999; Han and Lee, 2011; Insaustic *et al.*, 2001; Park, 2004). Since spoilage of meats is easily affected by the storage time and temperature, most spoilage studies of meat products are carried out by evaluating the microbiological and/or sensory quality of the product as a function of storage time (Nychas *et al.*, 2008). Although sensory and/or microbiological analyses widely utilized in assigning shelf-life of foods or troubleshooting problems with spoilage under storage, there are some disadvantages such as: the impossibility of automated measurements, lack of objectiveness, poor reproducibility, labor-inten-

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sive, and costly nature (Jos and Huis, 1996). Determination of the shelf-life or spoilage of meat and poultry products has been accomplished in recent years through the use of time temperature indicators (TTIs) (Rokka *et al.*, 2004; Smolander *et al.*, 2004; Vaikousi *et al.*, 2009).

TTIs are effective devices placed on the food package to monitor, record, and cumulatively indicate the effect of the full or partial temperature history of food products from manufacture to final consumption (Taoukis and Labuza, 1989). Operational principles of TTIs are based on mechanical, chemical, enzymatic, microbiological or photosensitive irreversible change, usually expressed as a visible response in the form of mechanical deformation, color development or color movement (Taoukis, 2001). Microbial TTIs are an indicator based on the acidification of the TTIs medium by selected lactic acid bacteria (LAB) that produce lactic acid as a result of carbohydrate fermentation, which induces a color change in a pH indicator. The LAB develop naturally in many perishable foods at temperatures and at growth rates that are close to spoilage bacteria and pathogens. Therefore, the use of LAB makes it possible to reflect the microbial degradation kinetics of foods on which the microbial TTIs are affixed (Ellouze *et al.*, 2008). For good example, Vaikousi *et al.* (2008) used *Lactobacillus sakei* to develop a prototype microbial TTI. In the present research, we manufactured microbial TTI using *Weissella cibaria* which had been classified as *Lactobacillus* spp. before reclassification. It was reported that *Weissella* species have no hygienic problems in the food application process (Kang *et al.*, 2006).

Generally, the visible response of TTIs must match the food quality loss due to growth or metabolic activities of spoilage bacteria in order to successfully apply microbial TTIs to food products. This study was conducted to investigate whether a relationship exists between visible color change of TTIs and the traditional indices for shelf-life/spoilage of ground beef during storage. Therefore, we manufactured microbial TTIs and measured OFD time, color, pH, VBN, AMB counts, and LAB counts of ground beef at different temperatures of 5, 10, 15, and 25°C, respectively.

Materials and Methods

Samples

The ground foreleg meat of the highest grade 1++ Hanwoo (Korean native cattle) was purchased from a local market in Seoul, Korea, for this study. Five grams of the sample was placed in a 50 mL Falcon conical tubes (FEG

352070, Becton Dickinson, Franklin Lakes, USA), kept in a deep freezer (DF 3524, Ilshin Lab. Co., Korea) with an average temperature of -35°C until defrosted in a refrigerator (GC-114HCMP, LG Electronics Inc., Korea) at 5±1°C for 2 h prior to analyses.

Manufacture of the microbial TTI

Weissella cibaria CIFP 009 (provided by Center for Intelligent Agro-Food Packaging, Dongguk University, Korea) is a psychrotrophic lactic acid bacterium used to manufacture the microbial TTI. *W. cibaria* CIFP 009 are selected whose behavior, depending on the temperatures and growth rates, corresponds to the growth kinetics of the microorganisms degrading the perishable foods. *W. cibaria* CIFP 009 were inoculated at 5 Log CFU/mL in a 50 mL centrifuge tube with 20 mL of de Man-Rogosa-Sharpe (MRS; Difco, USA) broth, which was then incubated at 37°C for 15 h. The strains were centrifuged (6860×g for 10 min, 4°C) and then washed twice with sterile distilled water. The cells from 10 centrifuge tubes were combined in a heap. To manufacture the microbial TTI, the collected cells were immediately mixed into sterile TTI base with 0.1 N NaOH to adjust pH to 7.0. TTI base was prepared according to Table 1.

Off-flavor detection (OFD) time

The sensory evaluation of the off-flavors in the defrosted samples was performed isothermally at four different storage temperatures (5, 10, 15, and 25°C). The sensory evaluation was conducted with appropriate time intervals and described the presence of off-flavors with respect to the sample storage time. Ten selected panelists (ages: 23-30, gender: 6 female and 4 male students from Dongguk Uni-

Table 1. Composition of microbial TTI base

Components	Concentration (g)
Glucose	30.0
Tryptone	5.0
Yeast extract	7.5
KH ₂ PO ₄	1.0
Tween 80	0.5
MgSO ₄ ·7H ₂ O	0.2
MnSO ₄ ·H ₂ O	0.05
Sodium acetate	5.0
Sodium citrate	5.0
Bromocresol purple	3.0
Methyl red sodium salt	1.0
Low melting agarose	10.0
Distilled water	1,000 ¹⁾

¹⁾mL

versity, Korea) were employed to evaluate the presence of off-flavors in the sample. The panelists practiced the off-flavor detecting method using a signal reference (stale samples that were stored at 30°C for 48 h) and noise reference (fresh samples). All evaluations were carried out in triplicate, since the sensory data on off-flavor detection were decided based on the subjective judgment of panelists. A dataset from the panelists was in binomial responses of either detected or not detected off-flavors. Because the sensory data acquired by the human senses involves uncertainty, logistic regression was performed on the relationship between the binomial dataset and the storage times (Eq. (1)).

$$\text{logit}(P_x) = \ln\left[\frac{P_x}{1-P_x}\right] = \beta_0 + \beta_1 x \quad (1)$$

where P_x is the probability of OFD, β_0 and β_1 regression constants, and x the storage time. The OFD time was presumed as the time at $P_x = 0.5$, which means that 50% of the panelist said there was off-flavors in the sample.

Determination of microbiological quality

Five grams of each sample was weighed aseptically and homogenized with 45 mL of sterile peptone buffered water (Difco, USA) using a purifier (Pursifier[®]; Microgen Bio-products Ltd., UK) for 180 s. Further decimal dilutions were made with the same diluents, and duplicates of at least three appropriate dilutions were plated on appropriate media: Petrifilm[™] Aerobic Count Plate (ACP; 3M, USA) incubated at 37°C for 48 h for aerobic mesophilic bacteria counts. Bromocresol purple plate count (BCP; Eiken Chemical Co., Japan) agar was anaerobically incubated at 30°C for 48 h (BBL Gas Pak Plus Anaerobic System; Becton Dickinson Microbiology Systems, USA) for enumeration of lactic acid bacteria counts. Colonies were counted, and the logarithms of the number of colony forming units (Log CFU) per gram sample were calculated.

Measurement of volatile basic nitrogen (VBN) content

VBN content of the sample was analyzed using Conway's method according to the method of KFSA (2002). Five grams of the sample was dispersed in 25 mL of distilled water and was homogenized for 5 min. The homogenate was filtered through a filter paper (Whatman No.1, Whatman International Ltd., England). The filtrate was made into weak acid with addition of 5% (v/v) sulfuric acid. One milliliter of the filtrate and saturated K_2CO_3

were put into the outer space of the Conway tool in each site and 1 mL of 0.01 N H_2SO_4 was added into the inner space of the Conway tool. The sealed Conway tool unit was shaken slowly to mix the filtrate and saturated K_2CO_3 and then incubated at 25°C for 60 min. After this, 10 il of Brunswik reagent (0.2 g of methyl red and 0.1 g of methylene blue in 300 mL ethyl alcohol) was added into the inner space for titration using 0.01 N NaOH. The VBN value was calculated by the following Eq. (2):

$$\begin{aligned} \text{VBN mg}\% (\text{mg}/100\text{g sample}) &= \\ &= 0.14 \times \frac{(b-a) \times f}{W} \times 100 \times d \end{aligned} \quad (2)$$

where W is the meat sample weight in grams, b is the volume of added 0.01 N NaOH in blank in mL, a is the volume of added 0.01 N NaOH in the sample in mL, f is the standard factor of 0.01N NaOH, and d is the dilution factor. All experiments were performed in triplicate.

pH and color measurement

Five grams of the sample was blended with 20 mL of distilled water for 60 s using a homogenizer (T65 D ULTRA-TURRAX[®], IKA[®], China). The pH values were measured immediately after the homogenization with a pH meter (S20 SevenEasy[™] pH, Mettler-Toledo International Inc., Korea). All determinations were performed in triplicate.

The CIE color L^* - (lightness), a^* - (redness), and b^* - (yellowness) values of the samples were determined using a colorimeter (Chroma meter CR-300, Konica Minolta Sensing Inc., Japan; illuminate C, calibrated with a white plate, $L^*=+97.09$, $a^*=0.26$, $b^*=1.84$). Measurements were taken at two random locations from both sides of three replicates.

Measurement of titratable acidity (TA) of the microbial TTI system

The TA of the microbial TTI system was measured isothermally at four different storage temperatures (5, 10, 15, and 25°C). Approximately 5.0 g of the TTI base was diluted with approximately 20 mL of sterilized distilled water and homogenized for 10 s. Added 5-6 drops of 1.0% phenolphthalein (w/v ethanol) to the sample and titrated with 0.1 N NaOH to a phenolphthalein end point or alternatively, to a pH of 8.2. TA was expressed as percentage of lactic acid, determined using the following Eq. (3):

$$\text{Lactic acid } (\%) = \frac{M \times N \times E}{S} \times 100 \quad (3)$$

where M is the volume of NaOH used, N is the molarity of NaOH, E is the equivalence factor (0.009) and S is the weight of sample. All experiments were performed in triplicate.

Measurement of color grade of the microbial TTI system

Digital images were made in order to obtain the color grade of the microbial TTI. The microbial TTIs were poured into a 6-Cell Cultureware (353502, Becton, Dickinson and Company, USA) of the same depths. The 6-Cell Cultureware was incubated at 37°C for 24 h. Digital images were taken with appropriate time intervals using a scanner (CLX-3185WK, Samsung, Korea). Photographic images were saved as RGB color images and then printed onto chromatic cardboards using a color photo printer (HP Designjet Z2100 44, Hewlett-Packard Development Co., USA). The visible color change of the microbial TTI system was observed isothermally at four different storage temperatures (5, 10, 15, and 25°C) on the basis of chromatic cardboard. Table 2 shows the RGB values of color grade of microbial TTIs. Measurements of color change were taken in the dark at controlled illuminance condition from three replicates.

Analysis of Arrhenius temperature dependence of quality indices for ground beef and response of microbial TTI


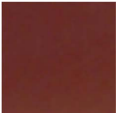


In an analysis of the temperature dependence of deteriorated quality of ground beef and response of TTI, the rate constants were determined on the basis of previous research of Byeon *et al.* (2009). We calculate the rate constants for the zero-order reactions using Eq. (4) and the rate constants for first-order reactions using Eq. (5).

$$y = k \cdot t + y_0 \quad (4)$$

$$\ln y = \ln y_0 + k \cdot t \quad (5)$$

where y is the experimental value, y_0 is the initial value, k is the reaction rate constant (1/h), and t is the storage

Table 2. RGB values for color grading system of microbial TTI

	Grade I	Grade II	Grade III	Grade IV
				
Red	79	104	153	207
Green	23	43	101	145
Blue	60	40	25	25

time. The activation energy was calculated by taking the natural logarithm on both sides of the Arrhenius function:

$$\ln k = \ln A + \left(\frac{-Ea}{R} \right) \left(\frac{1}{T} \right) \quad (6)$$

where k is the rate constant (1/h), A is a pre-exponential factor (1/h), Ea is the activation energy (kJ/mol), R is the ideal gas constant (8.314×10 kJ/K·mol), and T is the storage temperature (K). The reciprocal of OFD time, a rate, was regarded as the reaction constant (k), and then we used Eq. (6) to estimate the activation energy of OFD time. By plotting $\ln k$ and $1/T$, the values of Ea and A were read from the slope and the intercept.

Statistical analysis

The computation of off-flavor detection time and logistic regression of sensory evaluation were done using the Excel 2010 (version 14) for Microsoft Windows (Microsoft Corp., USA).

Results and Discussion

OFD time

The sensory evaluation of the off-flavors in the defrosted samples was performed with appropriate time intervals at the temperatures of 5, 10, 15, and 25°C. Logistic regression was executed on the binary sensory data of the presence/absence of off-flavors using Excel 2010. The OFD time, which is the time at $P_x = 0.5$, decreased with increasing storage temperature (Fig. 1). During storage at 5, 10, 15, and 25°C, the OFD time of ground beef was 170 h, 100 h, 48 h, and 12 h, respectively.

In previous research, Byeon *et al.* (2009) reported that the OFD times of sirloin were 151 h (5°C), 56 h (15°C), and 15 h (25°C), respectively. The OFD times in this study were shorter than those reported. This difference in OFD time could be the result of their production processes. Ground beef is more perishable than whole muscle cuts because, during grinding, the surface area of the beef is greatly increased, and any spoilage microorganisms present on the surface of the beef prior to grinding would be mixed throughout the entire ground beef, thereby shortening the OFD time. Off-flavors are strongly associated with the perception of meat palatability and are an important consumer quality attribute (Hilton *et al.*, 1998). Ammonia and ammonium salts generated by degradation of proteins, commonly as a result of microbial growth on stored meats, are major components of off-flavors (Cho, 1999; Player and Hultin, 1977). Moreover,

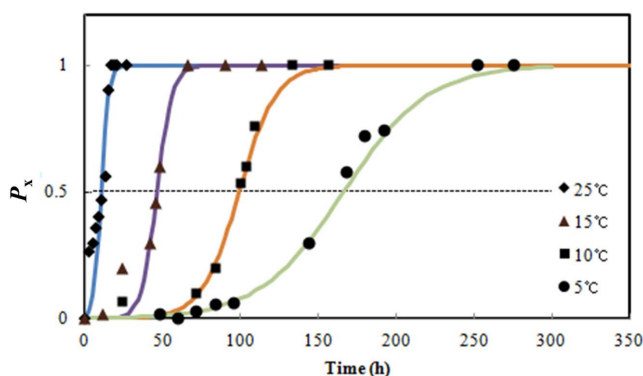


Fig. 1. Logistic regression of the probability (P_x) of off-flavor detection vs. storage time of the ground beef at different temperatures.

lipid oxidation of long-chain fatty acids in meats can contribute to off-flavors (Mottram, 1987).

Microbial spoilage of ground beef

Fig. 2 illustrates the changes in counts of AMB and LAB that occurred when the ground beefs were stored at four different storage temperatures (5, 10, 15, and 25°C). The growth rate of mentioned bacteria increased with increasing storage temperature in all experimental conditions.

Shin *et al.* (2006) demonstrated that the microbial spoilage of beefs began when microbial levels reached 6.0 to 7.0 Log CFU/g, and the level at which microbial spoilage occurred in beefs was 9 Log CFU/g. Thus for experiments on ground beef, the indicator the indicator level used for microbial spoilage was set at 7.0 Log CFU/g. The initial level of AMB and LAB in the samples immediately after preparation, was 5.59 Log CFU/g and 5.35 Log CFU/g, respectively. This result was within the range obtained by Lee *et al.* (2004): 5.2-5.5 Log CFU/g. During storage at 5, 10, 15, and 25°C, the AMB counts increased progressively throughout storage and reached 7.0 Log CFU/g after 167 h, 43 h, 29 h, and 8 h, respectively. After that, the AMB counts reached 9 Log CFU/g. The LAB counts followed a similar pattern.

VBN, pH and color characteristics of ground beef

In order to determine the physiochemical properties of ground beef, we measured the VBN content, pH value and CIE color value of the samples during storage at the temperatures of 5, 10, 15, and 25°C.

Fig. 3 shows the effect of temperature on VBN contents of the samples during storage at four different temperatures. When storage temperature increased, VBN content significantly increased in samples compared to their ini-

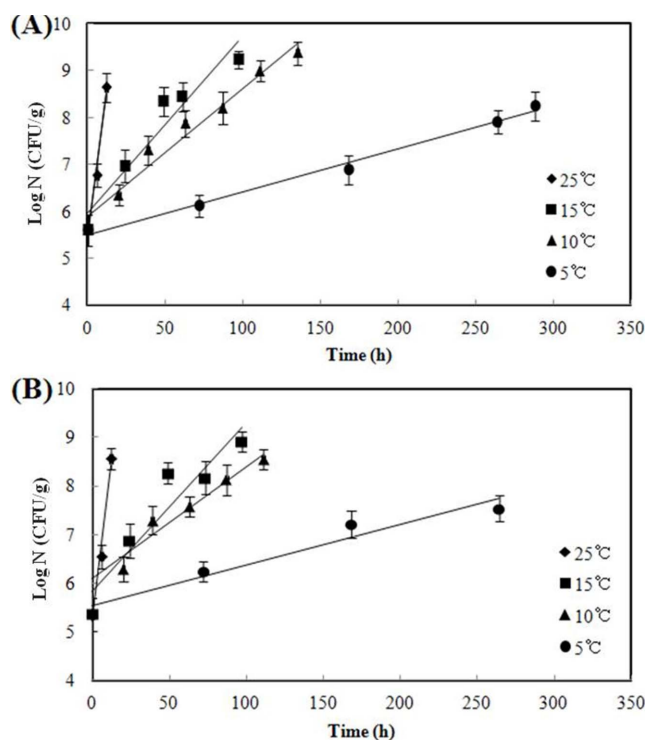


Fig. 2. Changes in aerobic mesophilic bacteria (A) and lactic acid bacteria (B) of the ground beef stored at different temperatures. Error bars indicate standard deviations ($n=3$).

tial content. The initial content of VBN was 4.38 mg%, which is similar to the value reported by Jeong *et al.* (2006). VBN content of meats increases as a spoilage of fresh meats since ammonia and amines are generated during storage as a result of deamination of amino acids and microbial or enzymatic decomposition of proteins (Al-Masri and Al-Bachir, 2007). Park *et al.* (1988) reported that a off-flavors can be detected when VBN content

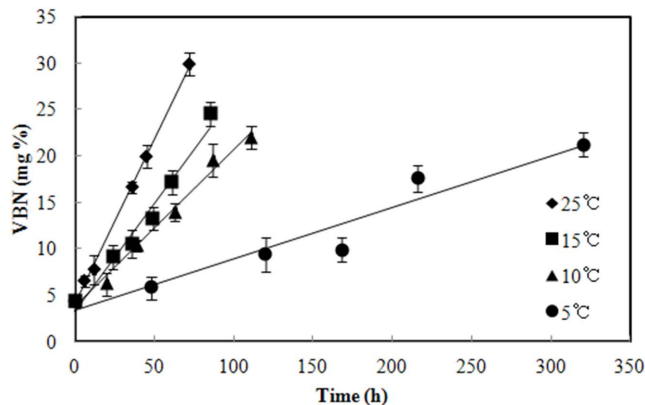


Fig. 3. Effect of temperature on volatile basic nitrogen contents of the ground beef during storage at four different temperatures. Error bars indicate standard deviations ($n=3$).

exceeds 15 mg%. In Korea, the upper limit of VBN is 20 mg% for fresh meat (KFDA, 2002). VBN contents in samples exceeded after 320 h (5°C), 111 h (10°C), 85 h (15°C), and 47 h (25°C) were <21.00 mg%, <22.00 mg%, <24.00 mg%, and <19.00 mg%, respectively.

Usually, after death, muscle becomes more acidic (pH decreases). The initial pH decline is caused by lactic acid accumulation during glycolysis and the later pH increment is caused by progressing alkalization due to the release of basic products of protein breakdown throughout the postmortem changes (Florek *et al.*, 2007). Typically, the pH decreases from a physiological pH of approximately 7.2 in the living muscle to a postmortem pH of approximately 5.5. As shown in Table 3, the initial pH value of ground beefs was 5.70, which then decreased from 5.70 to 5.54, and then increased suddenly. As the storage temperatures rose, changes in pH value occurred rapidly.

As previously reported (Andersen *et al.*, 1999), the pH value of meat can affect the shelf-life, color, tenderness, and eating quality of the meat. Therefore, pH measurements are important for determining meat quality and for giving a reasonably good indication of the final meat quality. Kim *et al.* (1996) observed that the meat with higher pH (above 5.9) was darker, more susceptible to bacterial spoilage and less flavored.

Average initial CIE color values of ground beefs were 48.02 ± 1.19 for L^* value (lightness), 19.52 ± 0.96 for a^* value (redness), and 9.79 ± 0.51 for b^* value (yellowness). When off-flavors were detected at 25°C (12 h), the CIE color L^* , a^* , and b^* values were 43.35 ± 0.82 , 13.54 ± 0.61 , and 7.64 ± 0.46 , respectively. The CIE color L^* , a^* , and b^* values after 48 h at 15°C were 44.92 ± 1.16 , 10.45 ± 0.70 , and 7.01 ± 0.84 ; those after 100 h at 10°C were 47.30 ± 0.75 , 11.17 ± 0.35 , and 7.58 ± 0.28 ; and those after 170 h at 5°C were 52.67 ± 0.31 , 7.68 ± 0.16 , and 8.63 ± 0.33 , respectively. On all of the experiments, the CIE color L^* , a^* , and b^* values decreased. Especially, the CIE color a^* value showed the biggest decrease. Similar results for the CIE color val-

ues of ground beefs were reported by Jung *et al.* (2009). Brewer and Harbers (1991) and Chen *et al.* (1999) previously reported that oxidation of oxymyoglobin affects the CIE color a^* value (redness) of red meats during storage and then redness was gradually decreased and did not affect the CIE color b^* value (yellowness). Our results were consistent with those reported above. The redness was gradually decreased during storage, and the yellowness showed little change during storage.

Changes in color and TA of the microbial TTI

The changes in the TA of TTI were monitored during storage at 5, 10, 15, and 25°C. As shown in Fig. 4, the rate of TA change increased with increasing storage temperature. The color change time at which a noticeable visual color change was observed, is shown in Fig. 5. In common with the results of TA, the color change time decreased with increasing storage temperature. We concluded that the visual color change of TTI occurred due to acid production from bacterial growth and metabolism in a TTI based manner.

Accurate color change time of microbial TTI was

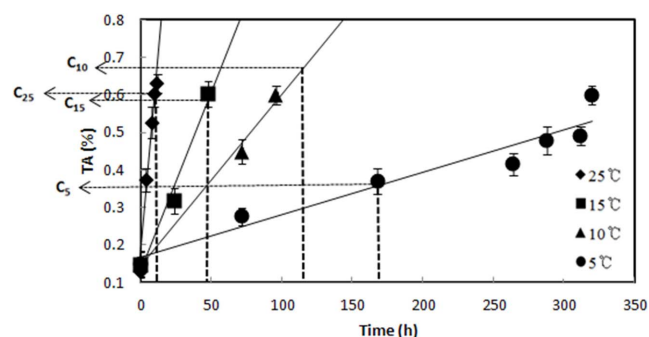


Fig. 4. Titratable acidity (TA) of the microbial TTI system during storage at different temperatures (C_X : TA concentration at off-flavor detection time stored at isothermal conditions. For example, C_{25} is a TA at off-flavor detection time stored at 25°C.). Error bars indicate standard deviations ($n=3$).

Table 3. Changes in pH of ground beefs during storage at 5, 10, 15, and 25°C

25 °C		15 °C		10 °C		5 °C	
Time (h)	pH	Time (h)	pH	Time (h)	pH	Time (h)	pH
0	$5.70 \pm 0.06^{1)}$	0	5.70 ± 0.01	0	5.70 ± 0.02	0	5.70 ± 0.05
6	5.64 ± 0.04	24	5.67 ± 0.04	20	5.69 ± 0.04	48	5.81 ± 0.05
12	5.52 ± 0.02	36	5.69 ± 0.07	39	5.72 ± 0.02	120	5.79 ± 0.02
36	5.54 ± 0.03	49	5.69 ± 0.02	63	5.65 ± 0.01	168	5.70 ± 0.03
45	6.23 ± 0.04	61	5.60 ± 0.02	87	5.61 ± 0.02	264	6.02 ± 0.02
72	6.68 ± 0.02	97	6.69 ± 0.02	111	6.35 ± 0.04	320	6.34 ± 0.03

¹⁾All values are mean±standard deviation ($n=3$).

observed (Fig. 5), and the TA values were read at which a visual color change was noted (Fig. 4). According to the above results and subsequent discussions, the relationship between each color grade and titratable acidity is shown in Fig. 6. The TA values of the 1st grade, 2nd grade, 3rd grade, and 4th grade were 0.0-0.146%, 0.146-0.247%, 0.247-0.416%, and 0.416-0.670%, respectively.

The difference of TA value as grade changed from first to second was smaller than those for third to fourth. It was thought that more acids are required for the decline of pH levels. In Fig. 4, C_x stands for the TA concentration of microbial TTI at OFD time in the samples stored at

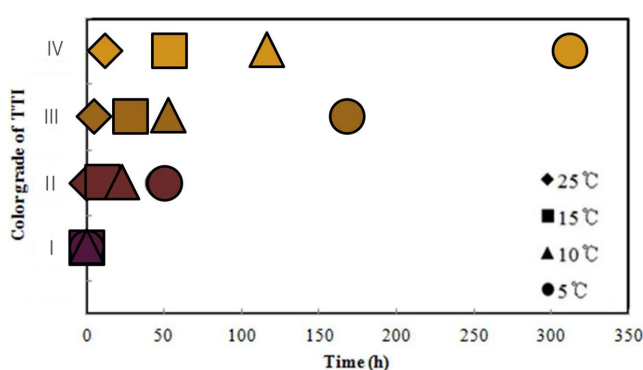


Fig. 5. Color grade change of the microbial TTI system during storage at different temperatures.

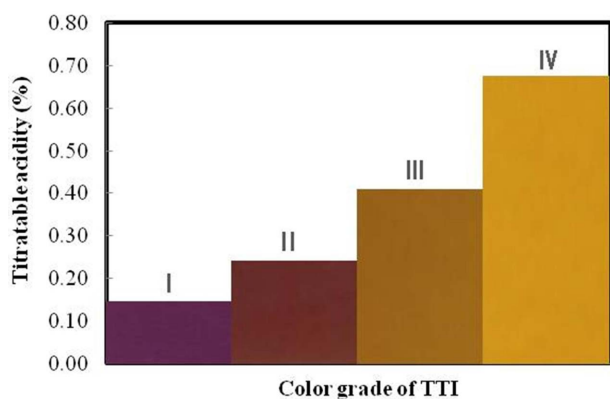


Fig. 6. Color grading system of microbial TTI according to titratable acidity.

isothermal conditions. The C_x values of TTI ranged from 0.5 to 0.7%, excluding TTI which was stored at 5°C. The results showed that the microbial TTI had a uniform color when off-flavors were detected in samples during storage at 10, 15, and 25°C, as expected.

Temperature dependence of quality indices for ground beef and response of microbial TTI

We analyzed the temperature dependence of TTI response and quality indices for ground beef to see if TTI response can represent the deteriorated quality of ground beef. The rate constant of TTI response and quality indices were calculated using Eq. (4) and (5). Under isothermal conditions, the activation energy (E_a) of quality indices for the beef deterioration kinetics and TA for the TTI response were calculated using Eq. (6). The activation energy (E_a) and the coefficient of determination (R^2) are shown in Table 4. The values of R^2 for the equation fitted with transformations of the same data were compared to determine the reaction order of deterioration kinetics for ground beef and TA of TTI.

As a result, the reaction order of VBN content, pH value, and microbial level was zero-order, first-order, and first-order reactions, respectively. In the case of a color of ground beefs, significant activation energy was hard to obtain due to the very low value of R^2 . The E_a values for the AMB count and LAB count were 110.35 kJ/mol and 116.65 kJ/mol, respectively. In this research, the activation energy for microbial levels in ground beef was within the range obtained by Vaikousi *et al.* (2009). They reported that the E_a values of *Lactobacillus sakei* in the minced beef products and the microbial TTI system were 111.90 kJ/mol and 106.90 kJ/mol, respectively. The E_a values for pH and VBN were 70.88 kJ/mol and 58.98 kJ/mol. The activation energy for VBN of ground beef is similar to those found by other authors in beef: 67.97 kJ/mol (Byeon *et al.*, 2009) and 66.70 kJ/mol (Han *et al.*, 2012). To estimate the activation energy for OFD time, the multiplicative inverse of OFD time was regarded as

Table 4. Activation energy (E_a) from Arrhenius' plots, and coefficient of determination (R^2) estimated by the fit of the linear regression

		Zero-order reaction		First-order reaction	
		E_a (kJ/mol)	R^2	E_a (kJ/mol)	R^2
Ground beef	Aerobic mesophilic bacteria	-	-	110.35	0.98
	Lactic acid bacteria	-	-	116.65	0.98
	VBN	58.98	0.84	-	-
	pH	-	-	70.88	0.92
TTI		104.36	0.91	106.22	0.96

the reaction constant (k), and then $\ln k$ values were plotted against $1/T$. The value of E_a for OFD time was 92.73 kJ/mol. The activation energy of TTI response (changes in TA) was 106.22 kJ/mol at both zero-order and first-order reactions. In particular, the E_a of microbial TTI was found to be closer to that of the microbial levels and OFD time. Taoukis *et al.* (1991) previously reported that typical E_a values for food quality losses due to chemical, physical, and biological changes ranged from 30 kJ/mol to 120 kJ/mol. TTIs are able to represent the food quality if they have similar temperature dependence (E_a) with the food spoilage mechanism. The activation energy for the chemical reaction of TTIs must be close to that of foods, at least within ± 25 kJ/mol (Taoukis, 2001). In this respect, microbial TTI could be a reliable indicator to represent the deteriorated quality of ground beefs due to microbial growth and off-flavor development during storage.

Relationship between the freshness of ground beef and TA of TTI connected with visual color change

In this research, freshness of ground beef is defined as follows: we assumed TA at OFD time as 0% freshness and TA at the start of storage as 100% freshness. These assumptions are described in Fig. 7. We also reproduced color grade of TTI related to TA change in Fig. 7. C_5 , C_{10} , C_{15} , and C_{25} were 0.37%, 0.60%, 0.60%, and 0.63% during storage at 5, 10, 15, and 25°C, respectively.

For example, at 25°C freshness of ground beef is a 100% if TA of TTI was 0.14%. On the contrary to this, freshness of ground beef is a 0% if TA of TTI was reached the arbitrary value of 0.63%. Using the result shown in Fig. 6, the TA of TTI in Fig. 7 was divided into four legions with

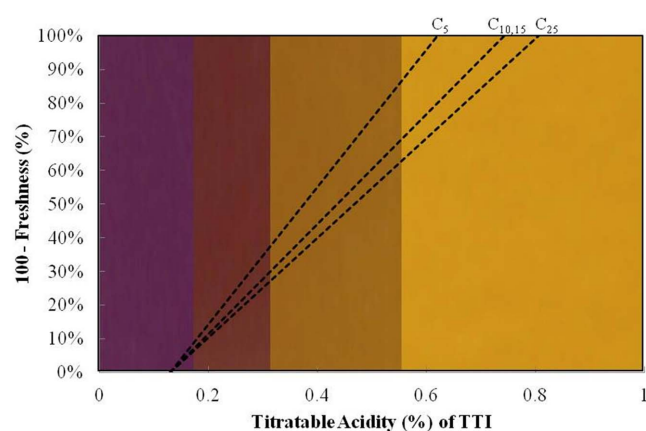


Fig. 7. Relationship between the freshness of ground beef and titratable acidity of TTI connected with visual color change at different temperatures. C_5 , C_{10} , C_{15} , and C_{25} were estimated using regression line of TAs shown in Fig. 4.

different color grades. Freshness of ground beef was less than 50% when the color grade of TTI was the fourth grade, regardless of storage temperature. As the difference between E_a value for OFD time and that for TTI response was less than 25 kJ/mol, freshness which is defined base on OFD time could be explained by the color of microbial TTI.

Quality indices of ground beef correspond to TTI color

Fig. 8 shows the relationship between the changes in the AMB LAB counts in the sample and TA of TTI in regard to visual color change. The TA of TTI, except in the case of storage temperature at 5°C, could represent the beef quality change due to the AMB counts. In the case of LAB, a certain LAB counts were obtained at each TA, regardless of storage temperature conditions. As we mentioned earlier, microbial TTI is able to represent the biological quality losses of ground beef due to the difference between E_a value for microbial levels in ground beef and E_a value for TTI less than 25 kJ/mol. Given that deteriorated quality of foods results from biological changes in foods, microbial TTI will be able to directly represent the food quality change because both food and TTI are under the same reaction system.

Changes in VBN value of ground beef stored at different temperatures corresponding to TA in relation to visual color change of TTI are shown in Fig. 8. Changes in color grade of TTI occurred quickly during storage at 15°C and 25°C, but the VBN values did not exceed 20 mg% when TTI color reached 4th grade. On the other hand, the VBN values reached the arbitrary value of 15 mg%, which is known as a detectable concentration of off-flavor by park *et al.* (1988), when TTI color reached 3rd grade since the color of TTI changed gradually for relatively long storage periods at 5°C and 10°C. When compared with the temperature dependence (E_a), microbial TTI did not seem to represent the quality change of ground beef because the difference between E_a for VBN (58.98 kJ/mol) and E_a for TTI was more than 25 kJ/mol. However, the VBN values had a certain relationship in two different temperature groups of low (5-10°C) and high (15-25°C) groups. Therefore, TTI can be used to represent the quality change of ground beef if they are applied to a restricted temperature range for application.

Conclusions

We manufactured microbial time-temperature indicator

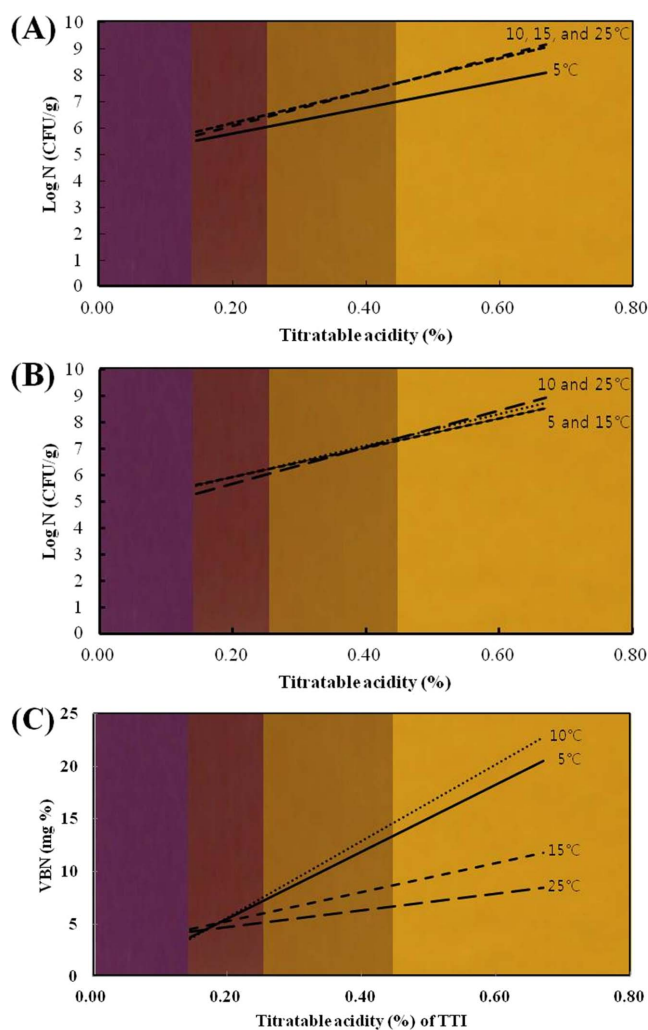


Fig. 8. Changes in aerobic mesophilic bacteria (A), lactic acid bacteria (B), and volatile basic nitrogen (C) in the ground beef stored at different temperatures corresponding to titratable acidity of TTI. —: 5°C, ----: 10°C, - - -: 15°C, and - · -: 25°C.

(TTI) in order to represent the quality change of ground beef and then measured the titratable acidity and color change of TTI. Quality indices such as off-flavor detection (OFD) time, color, pH, volatile basic nitrogen (VBN), aerobic mesophilic bacteria (AMB) counts, and lactic acid bacteria (LAB) counts were measured during storage at 5, 10, 15, and 25°C, respectively. As a result of temperature dependence analysis, the E_a value for AMB counts, LAB counts, VBN, pH, OFD time, and TTI response was 110.35 kJ/mol, 116.65 kJ/mol, 58.98 kJ/mol, 70.88 kJ/mol, 92.73 kJ/mol, and 106.22 kJ/mol, respectively. Especially, the E_a of microbial TTI was found to be closer to that of the microbial levels and OFD time. Therefore, microbial TTI could be a reliable indicator representing the deteriorated quality of ground beefs due to microbial

growth and off-flavor development during storage. Despite the potential of TTI to considerably contribute in improved food distribution and benefit the consumer with more useful shelf-life labeling their application up to now has failed to come up to the initial expectations. Factors such as cost, reliability and applicability have an effect on the reluctance of food manufacturers to adopt the TTI. In this sense, we are currently researching a complete scheme of translating TTI response to food products and simple and low-cost manufacturing method for microbial TTI.

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