Development of Maillard reaction based time-temperature indicator/integrator for application of agricultural and food process engineering

（農業および食品プロセス工学のための

メイラード反応に基づく

積算温度インジケータの開発）

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Table of Contents

Chapter 4 Application of a developed TTI into shipping and storage for vacuum-packaged beef

Chapter 5 Application of a developed TTI for visualization of heating state of processed foods

List of Tables

List of Figures

Chapter 1 Introduction

1.1. Importance of temperature management

Various agricultural processes such as harvest, distribution, storage are indispensable to deliver agricultural products to consumers. In those agricultural processes, temperature is one of the most important factor, which affects harvest timing, shelf-life, changes in quality during distribution or storage, and microbiological safety, etc.

Each crop has the minimum, maximum, and appropriate temperature. Appropriate temperature management during growing periods of crops promotes the growth and will be able to produce high quality agricultural products (Luo 2011). In the case of greenhouse cultivation, the temperature exposed can be controlled. Temperature difference, however, among the location of the crop in the greenhouse would occur by the nonuniformity of temperature distribution in greenhouse. As a consequence, the growth rate and optimal harvest timing differs among the growth position (Sharma et al. 1999; Bartzanas et al. 2002; Hattori et al. 2010). For this reason, although each crop is individually needed to manage temperature for, temperature management for individual crop is difficult in reality due to its troublesome work, cost, and etc. To realize individual temperature management for each crop, there is a need for a special easy-to-use tool.

Temperature management is also important factor during distribution and storage of food. Appropriate temperature management during distribution and storage will be able to deliver high quality crop to consumers. In general, the storage period of foods without spoilage or quality degradation will increase under low-temperature because low-temperature decreases the rate of biochemical and microbiological changes in foods

(Paull 1999). However, in the case of storage under freezing temperature, quality deterioration occurs in appearance and texture by freezing, despite the shelf-life significantly extends due to suppressed microbial growth and chemical changes (Barbosa-Cánovas et al. 2005; Aberoumand 2013). Therefore, raw agricultural commodities are needed to store under 0-7℃ for long-term storage with high quality (Laguerre et al. 2013). Prediction of shelf-life and quality degradation is needed even at low-temperature storage because the degradation of food under low-temperature storage is not stopped but just delayed. Moreover, there is non-uniformity of temperature distribution in the same storage space. Thus, confirmation and prediction of shelf-life and the integrated temperature for individual food is difficult using only confirmation of temperature (Mercier et al. 2017). There is a need for a special tool to provide information on shelf-life and/or integrated temperature of the stored food.

Temperature management plays an important role in food processing for microbiological safety. The Ministry of Health, Labor and Welfare of Japan released statistics that the accidents by food poisoning occurred 1,139 cases in 2016, the patients and the death people was 20,252 and 14 people, respectively. Cooking by higher enough temperature is one of methods for the preventing food poisoning (Terajima 2016). In addition, Bogard et al. (2013) reported that a majority (77%) of managers of the 385 restaurants in eight states of USA did not always measure the final cook temperatures of hamburgers with a thermometer, and twelve percent of the hamburgers were undercooked (cooked to 68.3℃). A survey by the United States Department of Agriculture (USDA) on hamburger cooking practices revealed that 20% of the participants cooked patties rare or medium rare (USDA 2002). The results of a telephone survey about 500 people in Ireland showed that the ratio for well-done, medium and rare of beef burgers was 87, 12, and 1 %, respectively (Duffy et al. 2006).

These results indicates that there would be a possibility of food poisoning by pathogenic bacteria such as Escherichia coli O157:H7. Thus, measurement and monitoring of internal temperature during cooking is recommended for the confirmation of exposure to high enough temperature in food. For this reason, a development of hardware has been needed to simply and visually monitor temperature history during thermal processing.

1.2. Time-temperature integrator/indicator

Confirmation of the temperature history during agricultural and food process is necessary. One of the hardware to realize the demands of temperature history monitoring and recording is time temperature integrator/indicators (TTIs) that has been attracted attention for long time. TTIs allow to indicate the remaining shelf-life of perishable products and the recording thermal history through the irreversible color change by the accumulative effect of time and temperature (Wang et al. 2015). Moreover, Taoukis and Labuza (2003) and Pavelková (2013) reported that TTIs are cost-effective and user-friendly devices to monitor, record, and translate the overall effect of temperature history. In those advantages, numerous TTIs have been studied.

TTIs are generally categorized as physical, chemical, and biological, based on the principles they make use of to achieve color change.

Physical-type diffusion-based TTIs as follows: Wanihsuksombat et al. (2010) reported that a lactic acid-based TTI prototype is based on the vapor diffusion of lactic acid could be applied to show the time–temperature history of some foods. Kim et al. (2016) studied that a prototype isopropyl palmitate (IPP) diffusion-based TTI system showed potential for monitoring the microbial quality of non-pasteurized angelica (NPA) juice based on temperature abuse. As the commercially available TTIs, 3M Monitor Mark® (3M Co., USA) is diffusion-based indicator label and is indicated by temperature-dependent permeation and a blue-dyed fatty acid ester diffusing along a wick. The response range of this TTI is various such as 48 hours for -15℃, 48 hours for 5℃, 48 hours for 10℃, and 1 weeks for 31℃, etc. (Pavelková 2013; Wang et al. 2015; 3M United States 2017). Time strips® (Timestrip UK Limited, UK) use a special porous membrane. The squeezing of a start button leads to move from the liquid to directly contacting the membrane, and then the liquid diffuses through the membrane in a consistent and totally repeatable way (Kuswandi et al. 2011). Time strips® also have various type and response range, but that response range is limited as 2 hours - 7 days despite available temperature range have a wide as -20 - 30℃ (Timestrip UK Ltd. 2017). Physical-type diffusion-based TTIs would be problematic because of the exudation of the color material and the fact that aging porous substances would have an adverse impact on safety and accuracy (Wang et al. 2015).

Chemical-type TTIs as follows: Ohta et al. (2008) reported that a color indicator based on the bacterial strain sk22 isolated from commercial cod developed for confirmation of temperature abuse in the cold chain. And the color in this indicator changed for 32-72 hours in 12℃. Kim et al. (2012a) studied a laccase based TTI prototype could be applied to predict losses of food quality ascribed to enzymatic changes, hydrolysis, and lipid oxidation. Murakami et al. (2012) reported that a simple indicator developed using red cabbage dye with sodium hydrogen carbonate, sodium carbonate and lactose and changed for 36, 48, and 96 hours in 12, 10, 4℃, respectively. Kuswandi et al. (2013) studied that a novel on-package color indicator based on bromophenol blue can be used for real time visual monitoring of freshness state of packaged guavas through the color indicator gradually changed color from blue to green after 5 days on room temperature. Yamamoto and Isshiki (2012) developed Maillard reaction-based TTI. This TTI was useful of chilled temperature distribution and has validated the accuracy of color change for alerting the growth of *Listeria monocytogenes* (Rokugawa and Fujikawa 2015). Park et al. (2013) reported that the laccase-based TTI including NaN_3 was composed two parts of an enzyme solution and a substrate solution and could be predicted the *Pseudomonas fragi* growth. As the commercially available TTIs, Fresh-Check® TTI (Temptime Co., USA) is using the resulting in a highly colored polymer by a solid state polymerization reaction. The polymer gradually darken depends on the color that tends to reflect the cumulative exposure to temperature. If the inner color is darker than the outer color, it means that the product has reached the end of shelf-life. The indicator has to be kept at -24℃ before application because indicators could be activated above the storage temperature (Pavelková 2013; Wang et al. 2015). But the range of use is limited due to the considering of use only for storage temperature. Other disadvantages of this TTI include the potential toxicity of polydiacetylene compounds when used in food products and the fact that its accuracy is affected by the selected compound and the presence of sunlight or bright direct light, which can accelerate the polymerization reaction (Wang et al. 2015). OnVu™ TTI (Ciba Specialty Chemicals & Fresh point Inc., Switzerland) is solid state reaction TTI, is based on the color change of photosensitive compounds and organic pigments by temperature. Additionally, this TTI is activated by the UV irradiation, changes from colorless to blue. Color change rate is proportional to temperature (Pavelková 2013; Wang et al. 2015). But it is considered that flexible using for various foods is limited because of the fixed change rate in the identical temperature.

Biological-type TTIs as follows: Vaikousi et al. (2009) reported that the color change in a microbial TTI prototype based on the growth and metabolic activity of a

Lactobacillus sakei strain was similar with the lactic acid bacteria (LAB) growth in fresh ground meat stored under modified atmosphere packed (MAP) conditions. Nopwinyuwong et al. (2010) studied that a novel colorimetric indicator label for monitoring freshness of intermediate-moisture dessert spoilage used pH-sensitive dyes, bromothymol blue, methyl red, and carbon dioxide (CO_2), and the indicator response correlates with microbial growth patterns. Kim et al. (2012b) reported that a microbial TTI using the *Weissella cibaria* CIFP 009 (psychrotrophic lactic acid bacterium) could be predicted accurately about aerobic mesophilic bacteria (AMB) counts, lactic acid bacteria (LAB) counts, and freshness. As the commercially available TTIs, Check Point® TTI (Vitsab A. B., Sweden) is based on a color change by the enzymatic system. The pH is decreased by a controlled enzymatic hydrolysis of a lipid substrate, and the pH decrease has occurred the color change of a pH indicator from deep green to bright yellow to orange-red (Kuswandi et al. 2011; Pavelková 2013). (eO)® TTI (CRYOLOG, France) is based on pH change by controlled microbial growth selected strains of lactic acid bacteria and is stored in a frozen state of -18℃ to prevent the bacterial growth. The color of this TTI is changed to red by temperature abuse, or when the product reaches its use by date (Pavelková 2013). Ellouze and Augustin (2010) reported that it can be used successfully as a quality management tool for the meat products. But it is also considered that flexible using for various foods is limited due to the difficulty of rate control for microbial growth.

Actually commercialized TTI shows Table 1-1.

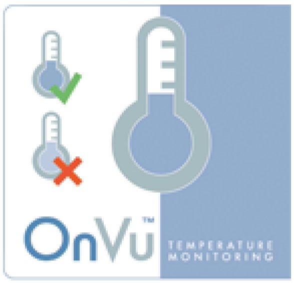
Table 1-1 The commercially available TTIs

3M Monitor Mark®

Time strips®

Fresh-Check® TTI

Check Point® TTI



OnVu™ TTI

(eO)® TTI

As such, various TTI were studied and commercialize. Although the TTIs will be useful for indicating the remaining shelf life of perishable products and confirmation of temperature history throughout storage, distribution and consumption, there are still a number of problems for TTIs such as migration of toxic substances, the inaccuracy of temperature monitoring, and high cost in commercial application. In addition, TTIs can monitor only surface temperature because that use around target food. Thus, the color change of TTIs do not directly reflect food quality but reflect environmental conditions. For this reason, further study will be needed to clarify the relationship between TTI and food quality. Furthermore, because the use of TTI in food process would lead to increase of production cost, suppressing the cost of TTI will be a critical element when people choose whether to use them or not (Wang et al. 2015).

Existing TTIs have limitation on flexibility at range of reaction temperature and/or reaction period. Since storage characteristics of each kind of food are very different even at the same storage temperature, ideal TTIs should have flexibility corresponding to each food characteristics by adjusting reaction rate. In fact, however, most of the existing TTIs cannot flexibly control the variation rate in same temperature and/or broaden available temperature range. If some special material used for the flexible TTIs, that would result in a rising cost and fall-off the usability. Consequently, the Maillard reaction was contemplated as the material with safety and flexibility.

1.3. Maillard reaction

Maillard reaction has long been known as one of food-related chemical reaction with color change (Ames 1998). Maillard reaction is a type of non-enzymatic browning and is form the brown nitrogenous polymers and co-polymers, known as Melanoidins by the reaction between a reducing sugar and a compound possessing a free amino group (Van 2006). This reaction was first described by Maillard (1912), but the first coherent scheme was put forward by Hodge (1953). And the Maillard reaction is related to aroma, taste, and color, in processes such as roasting of coffee and cocoa beans, baking of bread and cakes, toasting of cereals and the cooking of meat (Ames 1990; Martins et al. 2000).

Melanoidins as the produced by Maillard reaction during the processing and storage of foods become known to can preservation of the quality and safety of foods. Melanoidins can inhibit oxidation of unsaturated lipids and functional food ingredients, the growth of microorganisms, and prevent the spoilage and deterioration of foods, because of the antioxidant properties and antimicrobial activity (Wang et al. 2011).

Echavarría et al. (2012) reported that the melanoidins may have beneficial in antioxidant activity, human health, displaying in vivo antioxidant, antimicrobial and prebiotic activity in the intestine. Tamanna and Mahmood (2015) reported that Maillard reaction products have both positive and negative impacts such as antioxidants, bactericidal, antiallergenic, antibrowning, prooxidants, and carcinogens. A negative impact of Maillard reaction is mainly the existence of a carcinogen named acrylamide. However, acrylamide do not affect for TTI because that is formed by heating over 100℃ (Eden et al. 2002; Mottram, Wedzicha, and Dodson 2002; Stadler et al. 2002) .

Various studies have ported that the rate of Maillard reaction can be controlled by temperature, pH, kind of reactants and those concentrations. About this, Maillard (1912) reported that the reaction rate among the 150, 100, and 37 ℃ was faster with higher temperature. Ellis (1959) reported that the reaction rate increased at higher temperature and higher pH, and differed by the use of phosphates, a kind of sugar and amino acid. In particular, the order of reactivity for sugar was as follows; D-ribose > L-arabinose > D-xylose > D-galactose > lactose > D-glucose > maltose. Martins et al. (2000) reported that the reactivity of the sugar and amino group was also highly influenced by the temperature and pH, and was increased at the higher temperature and the higher pH. Sandwick et al. (2005) reported that the change in absorbance rate of reaction with glycine was 50 times faster with 0.05 M Ribose than those with glucose or ribose. Laroque et al. (2008) reported that the order of reactivity according to results of measurement of browning intensity, monitoring of the disappearance, and chromatographic data was as follows; fructose ≈ glucose < arabinose < xylose < ribose.

1.4. Research objectives

Various reaction rates can be obtained in Maillard reaction by the control of factors such as reaction temperature, pH, reactant, and concentration of reactant. Thus, the flexible property of Maillard reaction will be suitable for development of flexible TTIs. Moreover, this flexibility would be benefit low temperature food distribution management and other temperature management-related issues such as long-term storage and judgment of fruit ripening and harvesting times.

Herein, we aimed to maximize the applicability of the Maillard reaction for development of novel TTIs. To maximize the applicability of Maillard reaction, the color changes by the Maillard reaction were investigated under various concentration and temperature conditions, and a predictive model was developed for estimating the time required for these changes to be appeared as a function of temperature and reactant concentration. Furthermore, to verify the applicability of the developed Maillard reaction based TTI, the TTIs were examined on various agricultural and food processes such as distribution, storage, ripening, and cooking.

Chapter 2 Color variation characteristic of Maillard reaction

2.1. Introduction

The Maillard reaction has long been known as a food-processing-related chemical transformation accompanied by a color change (Ames, 1998). Despite mostly resulting in browning, this reaction can produce blue, green, and yellow colors at certain reaction temperatures, which was used by Yamamoto and Isshiki (2012) in their model Maillard reaction-based TTI. But this TTI was dedicated for low temperature management, the reaction rate was adjusted by a particular combination of reducing sugar and amino acid. However, numerous color variations would be realized by Maillard reaction, because the reaction rate can be controlled by adjusting combination of kind of reactants and those concentrations under various temperatures (Bell 1997; Benzing-Purdie, Ripmeester, and Ratcliffe 1985). These flexibilities of the color variation would enable to realize not only for managing low temperature food distribution but also for much more various fields relating to temperature management such as long-term storage, judgement of harvesting timing of fruits, and judgement of ripening some fruits, etc.

On the other hand, the design of TTIs for different food types requires the color change to be quantitatively evaluated by some measuring system. Furthermore, TTI color changes need to be correlated to food quality and/or safety changes to provide additional information on food distribution under the selected temperature conditions. Thus, quantitative evaluation of color change is indispensable for the development of flexible TTIs.

Therefore, we aimed to investigate color changes induced by the Maillard reaction under various conditions and establish a predictive model for estimating the time required for these changes to appear as a function of temperature and reactant concentration, using the analyzed data to develop novel TTIs.

2.2. Materials and methods

2.2.1. Reactants

D-xylose and glycine were used as reactants for the Maillard reaction due to exhibiting excellent reactivity and affording pronounced color changes (Ellis 1959; Laroque et al. 2008). But Maillard reaction speed using only D-xylose and glycine was slow at less than room temperature. Thus, Disodium hydrogen phosphate (Na_2HPO_4) was used as a reaction accelerator for fast reaction. The reactant concentrations show Table 2-1.

Table 2-1. Reaction conditions

Temperature	**Conditions**	**reactant**	**concentration (M)**
-0.5 ℃	Sub-zero reaction (For beef storage)	Xylose	1.0, 2.0, 3.0, 4.0, 5.0, 6.0
		Glycine	1.0, 1.5, 2.0, 2.5
		Na_2HPO_4	0.1
0, 5, 10, 15, 20, 25, 30 ℃	Normal reaction	Xylose	1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 7.0
		Glycine	1.0, 1.5, 2.0, 2.5
	Fast reaction (Exclusion 30℃)	Xylose	0.5, 1.0, 1.5, 2.0, 2.5
		Glycine	0.5, 1.0, 1.5, 2.0
		Na_2HPO_4	0.1, 0.2, 0.3, 0.4

Reaction solutions were prepared by mixing the D-xylose and glycine solutions as the volume ratio 1:1. During this process, D-xylose and glycine solutions were diluted by aqueous Na_2HPO_4 solutions of different concentrations, or by pure water at normal conditions. The thus obtained mixed reaction solutions were injected each 0.2 mL into the 96well microplate, and were reacted at incubator (CN-25C, MEE, TOKYO & SU240, ESPEC, OSAKA) of 0, 5, 10, 15, 20, and 25 ℃ until their color changed to black. Image of the solutions during Maillard reaction in 96well microplate show fig. 2-1. In microplate, the well of A~D and E~H were injected each same concentration because of repeatability and reproducibility.

Fig. 2-1 The solutions during Maillard reaction in 96well microplate

2.2.2. Analysis of color changes

Solution color variation was evaluated in the RGB color space (R (red), G (green), and B (blue)) by taking pictures with a digital camera (iPhone 5S, Apple, USA) at 12-h intervals. Fast reactions at 20 and 25 ℃ were sampled at 5-min intervals using smart-phone application (OS nap! Pro, Ver. 3.2, Justin Cegnar). Images were acquired at a resolution of 1920×1080 pixels, an f/2.2 aperture, and a 4.15-mm focal length, with a box blocking ambient light used to minimize lighting-related errors. A box for blocking ambient light was the size of 320×250×170 mm and supplied white paper on a floor for blocking influence by box color. And a source of light used only a flash in camera. For better performance, optimized exposure conditions were employed, i.e., the camera was used at an automatically set white balance and sensitivity (ISO 32). The captured images were analyzed using the R (Ver. 3.3.0) statistical environment running in the R studio (Ver. 0.99.491).

To compare reaction completion times under different conditions, a threshold value was required as a completion index. Herein, 0.10 in red color value was used as threshold values for the decision of completion time at normal reaction. And the threshold values for fast reaction were used 0.20, 0.15, and 0.15 at red, green and blue, respectively. Colors exhibiting appearing at lower RGB values than those threshold values appeared dark brown or black. Additionally, these values could also be used for timing purposes in the fabricated TTIs.

Color variation expressed by RGB value changes was described by a sigmoid function (Eq. 2-1):

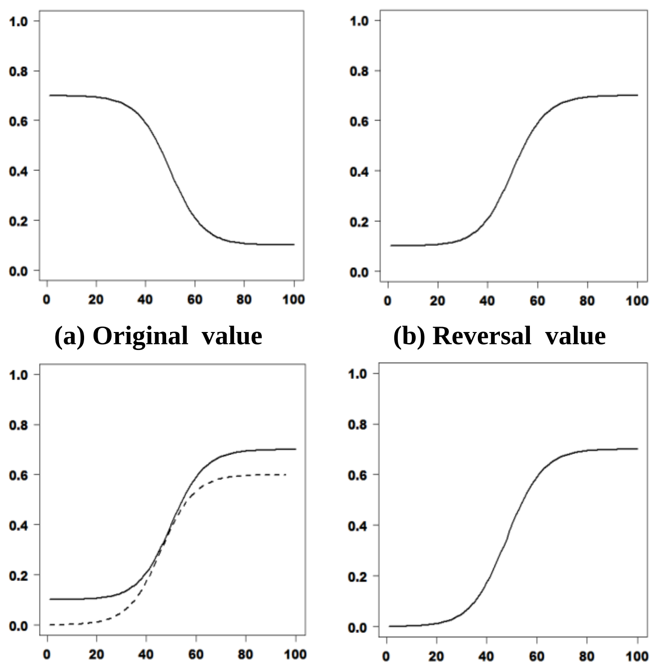
$$\text{R, G, or B value variation } (C_\text{v}) = \frac{\Delta C_\text{v}(\text{R, G, or B})}{1 + \exp\left(-r_{\max}(\text{time} - T_{\text{inf}})\right)} \qquad Eq.\ 2\text{-}1$$

where C_v color value, $r_{\max}$, time and T_{inf} denote RGB parameter changes, inflection point slope, reaction time, and inflection point time, respectively. Parameters for each condition were estimated using the non-linear least square method (R, nls package).

At this point, appropriate representations of the variation in the RGB values were fitted by model equation using the reverse of value by Eq. 2-2, and the offset of reversed value.

$$C'_v = -C_v + k \qquad \text{(Eq. 2-2)}$$

A Sigmoid function (Logistic function) show a “S” shape and commonly an increase curve of starting from 0. For this reason, Fitting by sigmoid function appeared some error because the observed color values show a decrease curve of starting from about 0.8. Thus, constant k in this study was decided 0.8. The original color variation was changed form a decrease curve to an increase curve by Eq. 2-2 (Fig. 2 (b)). And then, the reversal value was modified as deformation value of starting from 0 using dummy value by offset of original value (Fig. 2-2 (c)). This result show increased accuracy of fitting by sigmoid function.



(a) Original value **(b) Reversal value**

(c) Making of dummy and offset **(d) Modified value**

Fig. 2-2 The example of order for making modified value

The temperature dependence of all reaction conditions was expressed by Arrhenius function (Eq. 2-3):

$$\ln k = \frac{E_a}{RT} + \ln A \qquad \text{Eq. 2-3}$$

where k is μ_{max} (inflection point slope) of sigmoid function, E_a the activation energy, A is the pre-exponential factor, and R and T are ideal gas constant and absolute temperature (K), respectively. And slope (E_a/R) and pre-exponential factor were estimated using linear regression (Kim, Kim, and Lee 2012).

2.2.3. Predictive modeling of color change kinetics

Each parameter obtained by fitting the experimental data with Eq. 1 was described as a function of reactant concentration and reaction temperature with the help of multiple regression analysis (Eq. 2-4, Eq. 2-5). Eq. 2-4 was used at multiple regression analysis for normal reaction conditions. And Eq. 2-4 was used for fast reaction conditions. :

$$\text{Parameter } (\mu_{max}, T_{inf}, \text{or } \Delta\text{color value}) = a_0 + a_1 \times T + a_2 \times Xy + a_3 \times Gl + a_4 \times T^2 + a_6 \times Xy^2 \qquad \text{Ep. 2-4}$$

$$\text{Parameter } (\mu_{max}, T_{inf}, \text{or } \Delta\text{color value}) = b_0 + b_1 \times T + b_2 \times Xy + b_3 \times Gl + b_4 \times DH + b_5 \times T^2 + b_6 \times Xy^2 \qquad \text{Ep. 2-5}$$

Where a_i and b_i are coefficients, T is the reaction temperature (absolute temperature) and Xy, Gl, and DH are concentration of D-xylose, glycine, and Na_2HPO_4, respectively. And all parameter (r_{max}, T_{inf}, or Δcolor value), reaction temperature (T) and reactant concentration (Xy, Gl, DH) was transformed to a natural logarithmic value with the highest value in coefficient of determination of the regression analysis results. Additionally, data with temperature of 0, 5, 15 and 25 ℃ were used for predictive modeling. And predictive modeling was validated by data with temperature of 10 and 20 ℃.

2.3. Results and discussion

2.3.1. Characterization of color change under different conditions

The Maillard reaction between D-xylose and glycine showed a very pronounced color change as shown Fig. 2-3 from colorless through, yellow, light brown, and brown to black. In addition, the color change at the fast reaction using Na_2HPO_4 showed different to normal Maillard reaction from colorless through light blue, green, and brown to black (Fig. 2-4).

Fig. 2-3 Color change of Maillard reaction by D-xylose and Glycine

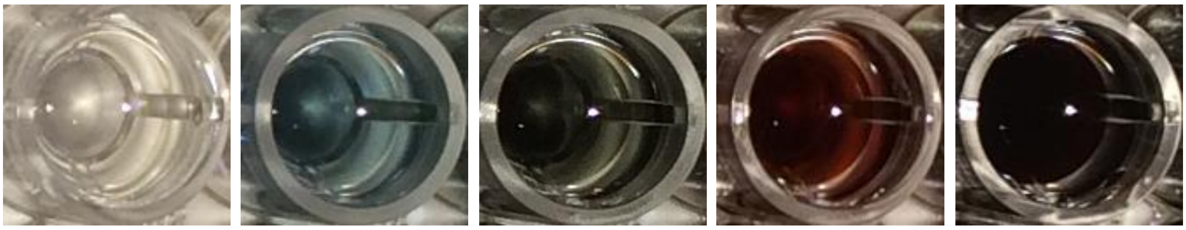


Fig. 2-4 Color change of Maillard reaction by D-xylose, Glycine and Na_2HPO_4

Commonly, such reactions, i.e., those between a reducing sugar and an amino acid, are accompanied by browning due to the generation of Melanoidin. However, the

Maillard reaction between glycine and D-xylose in slightly alkaline medium containing sodium bicarbonate as an accelerator initially generates three blue-colored pigments, denoted Blue-M1, Blue-M2, and Blue-M3 (Miura and Gomyo 1982).

Thus, the Maillard reaction between D-xylose and glycine solutions produced a wide range of colors thus being suitable for TTI development. The expression of various colors during the reaction enables users of TTI to recognize the temperature history by naked eyes. To be practically applicable, TTIs should exhibit color changes easily recognizable by the naked eye.

2.3.2. The quantitative evaluation of color change in Maillard reaction

Quantitative evaluation of color changes revealed that RGB values sigmoidally decreased under all conditions. The variation of color values was changed depending on using Na_2HPO_4. Figure 2-5 demonstrates representative observed/fitted color variations at 15 ℃ for Xy = 4.5 M and Gl = 2.0 M. The variation rate of the three color values (Red, Green, and Blue value) has clearly difference. The decrease of red color value was slowest than green value and blue value due to the expression of brown color by the generation of Melanoidin.

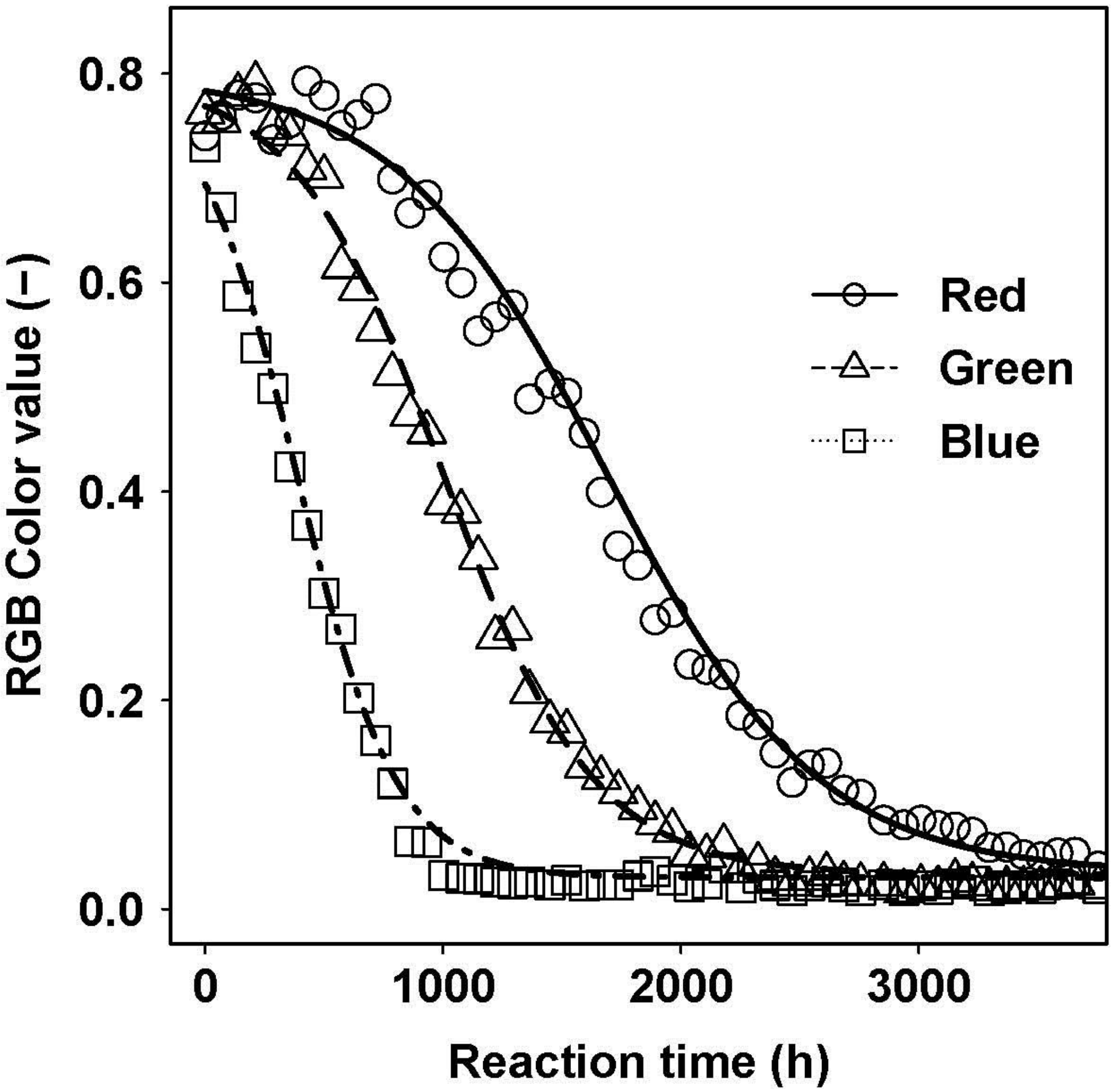


Fig. 2-5 A representation of the changes in the R (○), G (△), and B (□) values over time during normal Maillard reaction at 20°C in the condition of 4.5 M D-xylose and 2.0 M glycine. Solid, dashed, and dotted lines are illustrated fitted curves for R, G, and B, respectively.

Whereas, the variation rate of the three color values at conditions using Na_2HPO_4 was similar. Figure 2-6 demonstrates representative observed/fitted color variations at 10 ℃ for Xy = 1.0 M, Gl = 1.5 M, and DH = 0.3 M. As Fig. 2-6, the slope of the three color values was similar. Thus, the r_{max} and T_{inf} value at the results of fitting by Eq. 2-1 shown similar value, also. The fitted parameters of all conditions shows Table 2-2.

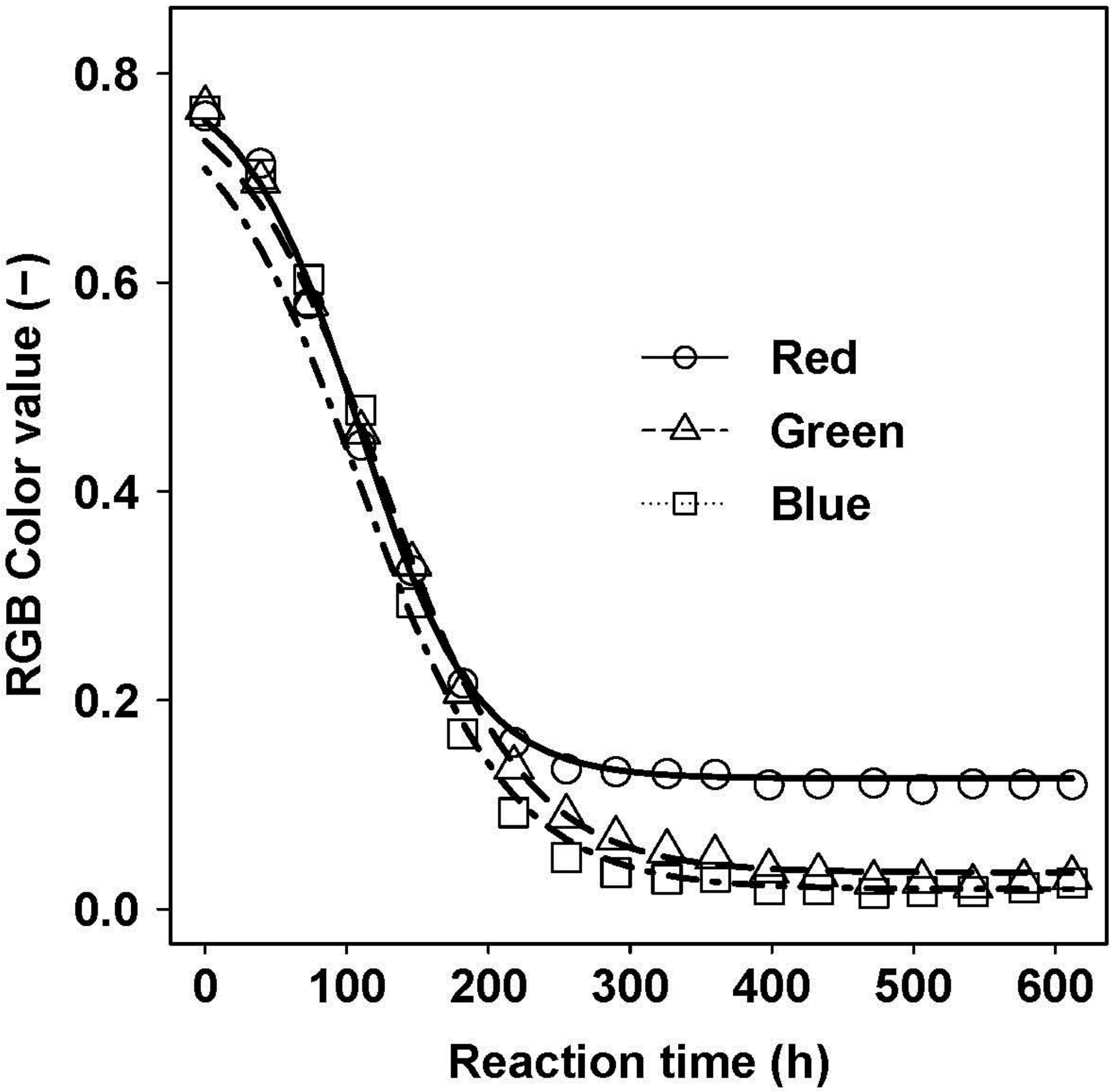


Fig. 2-6 A representation of the changes in the R (○), G (△), and B (□) values over time during fast Maillard reaction at 10°C in the condition of 1.0 M D-xylose, 1.5 M glycine, and 0.3 M Na_2HPO_4. Solid, dashed, and dotted lines are illustrated fitted curves for R, G, and B, respectively.

Table 2-2 (a) The fitted parameters (Ep. 1) at normal Maillard reaction of 5℃

Reactant concentration		Red				Green				Blue			
Xylose	Glycine	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0												
	1.5												
	2.0												
	2.5									0.7839	0.0163	186.9528	0.0410
1.5	1.0												
	1.5												
	2.0												
	2.5									0.7955	0.0185	113.1169	0.0321
2.0	1.0												
	1.5									0.7731	0.0329	75.2810	0.0424
	2.0									0.7866	0.0287	154.2107	0.0262
	2.5					0.7184	0.0116	238.3562	0.0338	0.7713	0.0268	90.6473	0.0229
2.5	1.0												
	1.5									0.7796	0.0126	169.8765	0.0255
	2.0									0.7835	0.0257	114.6133	0.0286
	2.5	0.8212	0.0091	369.0838	0.0402	0.7482	0.0152	203.6181	0.0235	0.7703	0.0364	83.5597	0.0149
3.0	1.0												
	1.5									0.7696	0.0169	139.3669	0.0206
	2.0									0.7824	0.0348	110.3735	0.0184
	2.5	0.7833	0.0104	312.9604	0.0395	0.7479	0.0182	170.7382	0.0273	0.7676	0.0411	75.2307	0.0117
3.5	1.0												
	1.5									0.7573	0.0177	113.6135	0.0254
	2.0									0.7766	0.0430	104.9982	0.0221
	2.5	0.7946	0.0126	255.7120	0.0377	0.7602	0.0233	152.7248	0.0235	0.7694	0.0423	64.8031	0.0124
4.0	1.0									0.8125	0.0104	235.6610	0.0241
	1.5					0.7529	0.0099	268.8092	0.0361	0.7691	0.0214	107.8477	0.0229
	2.0					0.8500	0.0112	246.9655	0.0459	0.7776	0.0342	81.8913	0.0136
	2.5	0.7461	0.0160	224.2222	0.0428	0.7719	0.0238	137.9582	0.0208	0.7723	0.0483	60.1580	0.0136
4.5	1.0									0.8016	0.0125	262.6927	0.0324
	1.5					0.7573	0.0119	244.1436	0.0336	0.7705	0.0246	98.0039	0.0197
	2.0					0.8002	0.0154	201.1066	0.0324	0.7807	0.0343	74.1623	0.0161
	2.5	0.7537	0.0155	189.3488	0.0307	0.7763	0.0254	123.3656	0.0229	0.7771	0.0439	50.2332	0.0182
5.0	1.0									0.7746	0.0172	205.6749	0.0254
	1.5					0.7665	0.0155	241.2774	0.0354	0.7733	0.0286	93.7093	0.0218
	2.0	0.7537	0.0163	289.6577	0.0488	0.7835	0.0189	174.0240	0.0256	0.7793	0.0514	78.2021	0.0153
	2.5	0.7538	0.0208	179.5359	0.0357	0.7796	0.0278	112.0799	0.0246	0.7778	0.0523	49.5958	0.0176
5.5	1.0									0.7827	0.0161	203.0597	0.0384
	1.5					0.8207	0.0111	234.0609	0.0610	0.7695	0.0340	100.0418	0.0340
	2.0	0.8254	0.0125	270.7164	0.0553	0.7815	0.0217	151.4443	0.0342	0.7820	0.0545	71.9829	0.0223
	2.5	0.7556	0.0226	166.8241	0.0486	0.7807	0.0369	118.2918	0.0393	0.7796	0.0525	47.5527	0.0210
6.0	1.0									0.7757	0.0140	173.7304	0.0334
	1.5	0.8470	0.0084	357.9057	0.0655	0.7885	0.0146	212.1147	0.0445	0.7729	0.0365	95.0313	0.0332
	2.0	0.7890	0.0136	236.9667	0.0432	0.7795	0.0234	137.3581	0.0342	0.7796	0.0458	63.7910	0.0233
	2.5	0.7636	0.0208	157.6842	0.0376	0.7898	0.0278	97.3919	0.0293	0.7838	0.0476	39.3201	0.0220
7.0	1.0									0.7914	0.0105	127.0733	0.0371
	1.5	0.8078	0.0090	293.1750	0.0558	0.7865	0.0153	179.8942	0.0416	0.7800	0.0263	65.4837	0.0310
	2.0	0.8177	0.0088	215.3046	0.0582	0.7697	0.0246	122.6631	0.0403	0.7807	0.0395	46.2695	0.0262
	2.5	0.7578	0.0241	131.5936	0.0397	0.7813	0.0367	89.2279	0.0326	0.7833	0.0488	33.0943	0.0222

Table 2-2 (b) The fitted parameters (Ep. 1) at normal Maillard reaction of 10°C

Reactant concentration		Red				Green				Blue			
Xylose	Glycine	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0												
	1.5									0.8145	0.0121	168.9603	0.0228
	2.0									0.7813	0.0226	135.6036	0.0199
	2.5									0.7798	0.0262	82.8052	0.0202
1.5	1.0									0.8782	0.0088	262.4455	0.0217
	1.5									0.8005	0.0140	110.0438	0.0251
	2.0					0.7627	0.0130	274.6216	0.0362	0.7795	0.0284	95.1217	0.0157
	2.5					0.7814	0.0171	167.1335	0.0302	0.7799	0.0324	59.1311	0.0216
2.0	1.0									0.8022	0.0157	212.8306	0.0179
	1.5									0.7850	0.0190	87.2814	0.0181
	2.0					0.7818	0.0184	195.9699	0.0282	0.7838	0.0281	65.3352	0.0258
	2.5					0.7793	0.0199	125.5595	0.0190	0.7770	0.0373	45.6819	0.0170
2.5	1.0									0.7943	0.0169	179.5644	0.0198
	1.5					0.7600	0.0151	220.0559	0.0268	0.7729	0.0257	88.8350	0.0145
	2.0	0.7617	0.0140	250.3152	0.0330	0.7786	0.0198	159.8019	0.0170	0.7823	0.0381	61.8579	0.0148
	2.5	0.7681	0.0202	193.6139	0.0346	0.7768	0.0232	109.9902	0.0201	0.7713	0.0432	41.9781	0.0170
3.0	1.0									0.7881	0.0199	163.4632	0.0226
	1.5	0.7603	0.0113	302.8810	0.0385	0.7595	0.0194	200.5803	0.0245	0.7671	0.0290	75.9330	0.0142
	2.0	0.7559	0.0180	219.1261	0.0319	0.7822	0.0200	131.4023	0.0172	0.7806	0.0374	52.0518	0.0113
	2.5	0.7662	0.0216	164.6028	0.0225	0.7684	0.0288	95.8244	0.0153	0.7642	0.0522	37.6828	0.0139
3.5	1.0									0.7862	0.0214	144.1770	0.0250
	1.5	0.7542	0.0128	248.9892	0.0346	0.7719	0.0212	177.9263	0.0232	0.7696	0.0323	68.1608	0.0127
	2.0	0.7702	0.0202	194.0337	0.0325	0.7850	0.0201	104.6641	0.0168	0.7799	0.0455	45.9754	0.0122
	2.5	0.7680	0.0206	133.6578	0.0211	0.7671	0.0347	85.3636	0.0134	0.7622	0.0699	36.7180	0.0125
4.0	1.0									0.7905	0.0163	107.1908	0.0248
	1.5	0.7641	0.0145	225.8835	0.0363	0.7813	0.0220	160.0150	0.0304	0.7748	0.0323	60.3013	0.0140
	2.0	0.7676	0.0237	181.6879	0.0312	0.7797	0.0245	95.8844	0.0172	0.7792	0.0443	38.0762	0.0137
	2.5	0.7709	0.0226	116.4892	0.0240	0.7716	0.0400	77.7254	0.0129	0.7678	0.0718	31.4208	0.0122
4.5	1.0									0.7757	0.0219	103.4222	0.0198
	1.5	0.7806	0.0142	200.7837	0.0340	0.7899	0.0184	125.9641	0.0224	0.7789	0.0359	55.3441	0.0135
	2.0	0.7703	0.0226	162.6312	0.0284	0.7752	0.0338	98.5620	0.0174	0.7774	0.0540	37.7610	0.0149
	2.5	0.7654	0.0334	111.0615	0.0231	0.7762	0.0468	71.3454	0.0142	0.7727	0.0856	29.4387	0.0122
5.0	1.0									0.7794	0.0235	101.7411	0.0219
	1.5	0.7791	0.0175	190.1525	0.0386	0.7860	0.0254	127.9842	0.0283	0.7822	0.0393	51.3852	0.0207
	2.0	0.7761	0.0236	146.7052	0.0361	0.7840	0.0312	91.5178	0.0334	0.7787	0.0666	37.9069	0.0147
	2.5	0.7676	0.0367	102.4487	0.0278	0.7776	0.0570	69.7439	0.0213	0.7767	0.0865	26.3136	0.0162
5.5	1.0									0.7789	0.0272	102.7101	0.0156
	1.5	0.7663	0.0218	189.1852	0.0495	0.7880	0.0257	129.9154	0.0457	0.7770	0.0470	55.8557	0.0220
	2.0	0.7710	0.0598	73.1146	0.0367	0.7819	0.0800	51.8937	0.0234	0.7805	0.1538	28.0728	0.0164
	2.5	0.7875	0.0259	82.8160	0.0464	0.7834	0.0495	59.7070	0.0349	0.7797	0.1001	26.3462	0.0203
6.0	1.0									0.7734	0.0330	101.7362	0.0210
	1.5	0.7744	0.0216	179.9589	0.0410	0.7853	0.0309	124.8111	0.0345	0.7831	0.0410	48.1476	0.0255
	2.0	0.7758	0.0318	118.1382	0.0430	0.7830	0.0487	80.7581	0.0336	0.7814	0.0850	35.9154	0.0197
	2.5	0.7817	0.0352	84.9194	0.0479	0.7848	0.0571	57.8974	0.0349	0.7821	0.1067	25.2034	0.0232
7.0	1.0					0.7797	0.0171	203.8619	0.0441	0.7781	0.0317	80.4163	0.0201
	1.5	0.7790	0.0216	160.7125	0.0410	0.7833	0.0322	108.7176	0.0312	0.7831	0.0568	44.7811	0.0157
	2.0	0.7686	0.0293	109.4978	0.0291	0.7755	0.0490	73.6987	0.0198	0.7791	0.0815	30.6010	0.0178
	2.5	0.7695	0.0392	78.6288	0.0405	0.7751	0.0605	52.0382	0.0242	0.7756	0.1096	20.9160	0.0139

Table 2-2 (c) The fitted parameters (Ep. 1) at normal Maillard reaction of 15℃

Reactant concentration		Red				Green				Blue			
Xylose	Glycine	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0									0.7736	0.0202	158.2995	0.0249
	1.5					0.7993	0.0105	248.1400	0.0422	0.7722	0.0270	79.2658	0.0214
	2.0	0.7777	0.0155	272.2694	0.0490	0.7948	0.0165	168.7242	0.0394	0.7667	0.0417	56.2872	0.0201
	2.5	0.7836	0.0154	192.4575	0.0430	0.7709	0.0250	113.9434	0.0202	0.7662	0.0759	52.2596	0.0256
1.5	1.0									0.7827	0.0236	113.7265	0.0186
	1.5					0.7842	0.0172	164.8611	0.0237	0.7796	0.0366	60.8410	0.0163
	2.0	0.7797	0.0184	188.1485	0.0385	0.7799	0.0261	117.8699	0.0228	0.7770	0.0650	47.0848	0.0134
	2.5	0.7834	0.0215	126.6367	0.0339	0.7773	0.0352	79.9866	0.0213	0.7729	0.0769	31.9954	0.0151
2.0	1.0	0.7576	0.0116	369.5818	0.0533	0.7558	0.0162	239.3707	0.0349	0.7688	0.0313	90.1346	0.0169
	1.5	0.7960	0.0117	189.8345	0.0381	0.7770	0.0211	118.1143	0.0206	0.7757	0.0386	41.8039	0.0162
	2.0	0.7692	0.0286	155.2845	0.0249	0.7743	0.0374	96.8730	0.0136	0.7739	0.0880	39.2934	0.0104
	2.5	0.7691	0.0292	91.0491	0.0224	0.7716	0.0496	61.1843	0.0195	0.7730	0.0698	19.4707	0.0171
2.5	1.0	0.7808	0.0123	302.9661	0.0545	0.7673	0.0213	207.0491	0.0255	0.7793	0.0298	72.3233	0.0182
	1.5	0.7714	0.0220	178.1117	0.0218	0.7749	0.0265	109.6029	0.0166	0.7692	0.0495	41.6818	0.0151
	2.0	0.7718	0.0292	116.8139	0.0202	0.7773	0.0425	75.1917	0.0120	0.7770	0.0839	30.8882	0.0100
	2.5	0.7598	0.0392	85.2861	0.0195	0.7618	0.0601	54.8173	0.0181	0.7579	0.1007	21.2834	0.0185
3.0	1.0	0.8042	0.0116	273.6539	0.0667	0.7669	0.0220	183.9918	0.0270	0.7676	0.0287	60.0360	0.0185
	1.5	0.7719	0.0256	153.8245	0.0178	0.7743	0.0331	93.5273	0.0148	0.7719	0.0530	36.1745	0.0122
	2.0	0.7651	0.0378	101.7059	0.0182	0.7739	0.0457	62.8448	0.0131	0.7720	0.0774	24.3196	0.0116
	2.5	0.7674	0.0433	68.6057	0.0168	0.7699	0.0649	45.1145	0.0119	0.7669	0.1136	18.6097	0.0119
3.5	1.0	0.7747	0.0162	235.1980	0.0547	0.7739	0.0253	164.3402	0.0194	0.7763	0.0326	47.3036	0.0145
	1.5	0.7748	0.0196	114.1890	0.0212	0.7721	0.0366	80.0174	0.0119	0.7687	0.0604	31.6228	0.0113
	2.0	0.7690	0.0364	79.3906	0.0167	0.7748	0.0510	49.6101	0.0149	0.7756	0.0816	19.0206	0.0123
	2.5	0.7661	0.0437	56.6841	0.0160	0.7665	0.0808	39.8700	0.0136	0.7652	0.1213	15.4669	0.0112
4.0	1.0	0.7694	0.0162	212.5966	0.0454	0.7737	0.0243	149.0651	0.0175	0.7739	0.0366	44.6852	0.0159
	1.5	0.7713	0.0290	98.2318	0.0177	0.7739	0.0472	68.8905	0.0151	0.7718	0.0720	26.9214	0.0125
	2.0	0.7651	0.0442	72.3243	0.0165	0.7727	0.0566	42.7887	0.0161	0.7743	0.0916	17.0990	0.0123
	2.5	0.7663	0.0578	55.0879	0.0163	0.7694	0.1015	39.4210	0.0116	0.7693	0.1368	14.8239	0.0096
4.5	1.0	0.7586	0.0210	201.5693	0.0370	0.7688	0.0258	130.3112	0.0198	0.7699	0.0464	46.2386	0.0153
	1.5	0.7733	0.0295	96.3769	0.0204	0.7751	0.0512	67.8387	0.0147	0.7746	0.0816	27.7640	0.0102
	2.0	0.7598	0.0529	67.3287	0.0173	0.7664	0.0759	41.7158	0.0157	0.7695	0.1141	16.0867	0.0139
	2.5	0.7684	0.0617	46.3661	0.0167	0.7718	0.0994	32.8560	0.0116	0.7714	0.1754	13.8714	0.0103
5.0	1.0	0.7730	0.0241	182.7156	0.0325	0.7783	0.0316	122.9157	0.0204	0.7770	0.0653	50.1871	0.0128
	1.5	0.7726	0.0386	95.3999	0.0226	0.7759	0.0568	63.3579	0.0172	0.7761	0.0838	24.5350	0.0137
	2.0	0.7724	0.0570	66.1492	0.0177	0.7755	0.0921	43.8800	0.0137	0.7768	0.1600	19.1005	0.0092
	2.5	0.7710	0.0821	46.7092	0.0186	0.7743	0.1347	33.3004	0.0152	0.7753	0.1624	11.8082	0.0142
5.5	1.0	0.7755	0.0255	164.3891	0.0337	0.7793	0.0400	116.2955	0.0244	0.7809	0.0633	46.5262	0.0113
	1.5	0.7780	0.0374	90.3028	0.0396	0.7794	0.0624	62.8286	0.0314	0.7788	0.1156	27.3723	0.0191
	2.0	0.7749	0.0641	67.1467	0.0179	0.7778	0.0988	44.6029	0.0123	0.7783	0.1569	19.0418	0.0091
	2.5	0.7754	0.0863	46.2510	0.0263	0.7794	0.1024	30.6865	0.0250	0.7781	0.1987	13.1467	0.0145
6.0	1.0	0.7819	0.0232	157.1978	0.0418	0.7818	0.0336	104.0023	0.0305	0.7802	0.0685	43.6965	0.0173
	1.5	0.7793	0.0345	73.8024	0.0304	0.7832	0.0504	52.3509	0.0355	0.7812	0.1268	26.1408	0.0181
	2.0	0.7737	0.0708	61.0936	0.0234	0.7785	0.0962	40.7259	0.0218	0.7793	0.1669	17.7800	0.0131
	2.5	0.7771	0.0972	42.6264	0.0200	0.7798	0.1205	28.3310	0.0148	0.7805	0.2003	11.5795	0.0109
7.0	1.0	0.7765	0.0215	132.1926	0.0416	0.7766	0.0391	89.2172	0.0285	0.7797	0.0601	31.1922	0.0147
	1.5	0.7704	0.0454	70.6444	0.0255	0.7746	0.0712	48.2646	0.0190	0.7791	0.1087	19.4860	0.0135
	2.0	0.7723	0.0807	49.5532	0.0213	0.7778	0.0979	32.2167	0.0152	0.7820	0.1664	13.7302	0.0106
	2.5	0.7734	0.0939	35.0422	0.0203	0.7768	0.0889	16.5536	0.0236	0.7811	0.2291	9.8075	0.0097

Table 2-2 (d) The fitted parameters (Ep. 1) at normal Maillard reaction of 20°C

Reactant concentration		Red				Green				Blue			
Xylose	Glycine	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0									0.7726	0.0305	73.4148	0.0254
	1.5	0.7708	0.0174	183.6858	0.0403	0.7742	0.0243	118.3503	0.0204	0.7771	0.0499	33.2053	0.0149
	2.0	0.6870	0.0451	138.8821	0.0429	0.7777	0.0386	92.9108	0.0338	0.7694	0.1050	28.4289	0.0153
	2.5	0.7652	0.0369	98.0406	0.0341	0.7748	0.0516	58.6059	0.0219	0.7738	0.1245	18.4839	0.0118
1.5	1.0									0.7782	0.0466	56.8089	0.0166
	1.5	0.7650	0.0279	135.5237	0.0359	0.7830	0.0306	78.6622	0.0261	0.7807	0.0618	23.1541	0.0142
	2.0	0.7720	0.0424	104.4407	0.0363	0.7814	0.0528	66.5775	0.0231	0.7767	0.1490	22.2209	0.0114
	2.5	0.7745	0.0493	67.0031	0.0265	0.7779	0.0645	36.3665	0.0208	0.7775	0.1625	14.8262	0.0133
2.0	1.0	0.7791	0.0197	199.0381	0.0427	0.7827	0.0252	131.8735	0.0242	0.7789	0.0587	41.4369	0.0138
	1.5	0.7717	0.0298	93.0926	0.0213	0.7755	0.0438	58.9910	0.0204	0.7748	0.0888	18.2959	0.0115
	2.0	0.7756	0.0521	77.0951	0.0337	0.7758	0.0830	50.1885	0.0259	0.7756	0.2053	18.7138	0.0119
	2.5	0.7636	0.0659	47.0607	0.0193	0.7726	0.1039	28.7050	0.0151	0.7738	0.1795	10.2860	0.0123
2.5	1.0	0.7901	0.0217	164.4747	0.0420	0.7804	0.0322	113.7011	0.0260	0.7697	0.0699	35.6874	0.0165
	1.5	0.7777	0.0362	83.8417	0.0333	0.7749	0.0634	59.4940	0.0199	0.7712	0.1323	20.8343	0.0149
	2.0	0.7710	0.0652	63.7753	0.0322	0.7785	0.0814	37.0837	0.0181	0.7757	0.2360	16.2357	0.0117
	2.5	0.7650	0.0833	43.1093	0.0210	0.7720	0.1283	26.9484	0.0183	0.7685	0.2689	11.1666	0.0123
3.0	1.0	0.7822	0.0236	132.8518	0.0264	0.7821	0.0321	87.7422	0.0223	0.7772	0.0791	30.6276	0.0128
	1.5	0.7679	0.0489	73.4331	0.0265	0.7706	0.0726	48.5925	0.0184	0.7649	0.1718	18.8965	0.0152
	2.0	0.7651	0.0778	53.8268	0.0284	0.7759	0.1104	32.4464	0.0175	0.7768	0.2077	12.6091	0.0130
	2.5	0.7664	0.1014	36.9381	0.0232	0.7720	0.1428	22.3456	0.0223	0.7653	0.3070	9.6097	0.0140
3.5	1.0	0.7759	0.0281	116.8964	0.0278	0.7802	0.0383	77.6437	0.0247	0.7766	0.0960	29.3518	0.0104
	1.5	0.7570	0.0579	65.7003	0.0258	0.7645	0.0756	40.1965	0.0196	0.7630	0.1876	16.5974	0.0125
	2.0	0.7687	0.0874	45.9308	0.0230	0.7759	0.1433	29.9986	0.0156	0.7760	0.2238	11.3568	0.0122
	2.5	0.7634	0.1139	32.1365	0.0233	0.7686	0.1726	20.0174	0.0221	0.7627	0.3872	9.1658	0.0137
4.0	1.0	0.7632	0.0373	109.9981	0.0292	0.7741	0.0513	75.5321	0.0264	0.7734	0.1111	27.2621	0.0125
	1.5	0.7690	0.0652	60.0127	0.0278	0.7732	0.0918	36.8024	0.0222	0.7719	0.1813	14.3887	0.0138
	2.0	0.7603	0.1050	41.1024	0.0232	0.7737	0.1525	26.7491	0.0169	0.7733	0.2730	11.0684	0.0106
	2.5	0.7597	0.1461	29.1710	0.0249	0.7687	0.2292	19.2845	0.0163	0.7657	0.3602	8.1171	0.0129
4.5	1.0	0.7679	0.0362	102.3279	0.0331	0.7733	0.0552	70.5137	0.0277	0.7719	0.1155	24.7914	0.0139
	1.5	0.7729	0.0664	51.0394	0.0268	0.7771	0.1012	32.4847	0.0233	0.7758	0.1911	12.9303	0.0136
	2.0	0.7647	0.1087	38.0334	0.0259	0.7726	0.1790	25.4246	0.0186	0.7721	0.2741	9.9684	0.0127
	2.5	0.7646	0.1677	24.5189	0.0228	0.7759	0.2329	16.7857	0.0162	0.7719	0.3885	7.0839	0.0114
5.0	1.0	0.7706	0.0432	94.5652	0.0321	0.7782	0.0583	63.3260	0.0273	0.7760	0.1296	23.8723	0.0117
	1.5	0.7701	0.0915	41.0454	0.0253	0.7783	0.1328	27.4878	0.0188	0.7774	0.2744	12.4726	0.0120
	2.0	0.7739	0.1114	34.6255	0.0292	0.7752	0.2124	23.7340	0.0166	0.7752	0.3264	9.7648	0.0090
	2.5	0.7637	0.1989	23.7340	0.0276	0.7806	0.2405	15.7090	0.0156	0.7766	0.3823	6.5737	0.0141
5.5	1.0	0.7880	0.0380	86.8555	0.0372	0.7832	0.0581	58.8406	0.0303	0.7791	0.1135	20.6208	0.0150
	1.5	0.7785	0.0719	41.6571	0.0450	0.7866	0.0961	28.5588	0.0489	0.7809	0.1787	11.8062	0.0226
	2.0	0.7738	0.1126	29.4339	0.0312	0.7814	0.1729	19.9642	0.0232	0.7785	0.3040	8.6430	0.0135
	2.5	0.7840	0.1400	21.5898	0.0495	0.7829	0.2552	15.4145	0.0342	0.7793	0.4589	7.0695	0.0237
6.0	1.0	0.7789	0.0498	86.4168	0.0328	0.7821	0.0642	56.3094	0.0244	0.7788	0.1451	21.5033	0.0134
	1.5	0.7743	0.0831	40.6378	0.0330	0.7819	0.1399	29.0544	0.0272	0.7800	0.2329	12.4070	0.0165
	2.0	0.7739	0.1397	29.5639	0.0323	0.7796	0.2226	20.0836	0.0208	0.7793	0.3909	9.0960	0.0110
	2.5	0.7754	0.1889	20.5174	0.0305	0.7828	0.2664	14.1966	0.0223	0.7797	0.4892	6.6084	0.0171
7.0	1.0	0.7781	0.0465	70.8231	0.0422	0.7788	0.0762	49.8362	0.0280	0.7809	0.1475	18.0116	0.0151
	1.5	0.7687	0.1109	38.5133	0.0284	0.7815	0.1336	24.7228	0.0238	0.7826	0.1989	9.7106	0.0170
	2.0	0.7707	0.1473	24.7373	0.0278	0.7789	0.2118	16.6239	0.0181	0.7802	0.3569	7.1785	0.0129
	2.5	0.7719	0.2144	18.0794	0.0226	0.7804	0.3047	12.8353	0.0153	0.7804	0.6285	6.1663	0.0135

Table 2-2 (e) The fitted parameters (Ep. 1) at normal Maillard reaction of 25℃

Reactant concentration		Red				Green				Blue			
Xylose	Glycine	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.7142	0.0524	166.6561	0.0431	0.7730	0.0476	126.3120	0.0350	0.7684	0.0666	41.3130	0.0166
	1.5	0.7431	0.0583	101.0130	0.0316	0.7621	0.0608	70.0409	0.0231	0.7611	0.1406	21.8947	0.0169
	2.0	0.7234	0.0813	70.8007	0.0332	0.7684	0.0768	46.8266	0.0293	0.7607	0.2169	16.0929	0.0168
	2.5	0.7679	0.0750	50.6463	0.0307	0.7693	0.1095	30.6715	0.0201	0.7729	0.2209	9.6246	0.0164
1.5	1.0	0.7434	0.0522	135.2053	0.0423	0.7851	0.0471	99.6719	0.0400	0.7724	0.0901	29.4179	0.0142
	1.5	0.7804	0.0560	73.4434	0.0303	0.7804	0.0675	49.6377	0.0256	0.7742	0.1651	16.2519	0.0151
	2.0	0.7763	0.0796	52.9291	0.0287	0.7762	0.1038	32.6366	0.0194	0.7760	0.2749	11.5796	0.0126
	2.5	0.7665	0.1117	36.9574	0.0265	0.7726	0.1566	23.0419	0.0205	0.7727	0.3921	8.8926	0.0146
2.0	1.0	0.7529	0.0568	107.1066	0.0331	0.7721	0.0585	76.9203	0.0228	0.7681	0.1173	22.5504	0.0137
	1.5	0.7807	0.0527	50.1552	0.0297	0.7603	0.0873	32.4908	0.0231	0.7599	0.1931	10.4971	0.0185
	2.0	0.7685	0.1034	40.5161	0.0243	0.7742	0.1431	25.3398	0.0150	0.7763	0.2987	9.2075	0.0116
	2.5	0.7629	0.1346	24.6211	0.0200	0.7680	0.2162	16.2838	0.0148	0.7693	0.4693	6.3457	0.0128
2.5	1.0	0.7589	0.0517	89.4581	0.0211	0.7685	0.0585	61.8462	0.0130	0.7647	0.1514	20.3050	0.0095
	1.5	0.7532	0.0799	43.3276	0.0200	0.7570	0.1129	27.1205	0.0194	0.7559	0.2327	9.7975	0.0158
	2.0	0.7679	0.1190	32.1074	0.0211	0.7742	0.1803	21.1102	0.0127	0.7761	0.3196	7.7731	0.0112
	2.5	0.7602	0.1775	21.2263	0.0189	0.7670	0.2313	12.9647	0.0170	0.7669	0.5214	5.7334	0.0142
3.0	1.0	0.7658	0.0559	74.1660	0.0186	0.7794	0.0565	47.0341	0.0216	0.7724	0.1249	14.3622	0.0142
	1.5	0.7565	0.0879	35.3100	0.0255	0.7615	0.1349	22.8335	0.0188	0.7609	0.3174	9.3945	0.0148
	2.0	0.7693	0.1203	25.5106	0.0197	0.7737	0.2048	17.7443	0.0117	0.7748	0.3286	6.5888	0.0110
	2.5	0.7563	0.1841	15.7662	0.0171	0.7714	0.2695	10.4004	0.0145	0.7698	0.4213	3.8267	0.0143
3.5	1.0	0.7808	0.0505	60.1298	0.0203	0.7763	0.0660	38.2376	0.0181	0.7745	0.1380	12.0565	0.0133
	1.5	0.7428	0.1092	29.3969	0.0222	0.7595	0.1594	19.6232	0.0150	0.7626	0.2509	6.6703	0.0135
	2.0	0.7688	0.1462	21.1654	0.0149	0.7741	0.2115	13.8597	0.0119	0.7752	0.3437	5.1481	0.0103
	2.5	0.7615	0.1929	13.1585	0.0179	0.7680	0.3462	9.4809	0.0138	0.7685	0.4534	3.3590	0.0148
4.0	1.0	0.7757	0.0581	56.2843	0.0207	0.7712	0.0810	36.1663	0.0194	0.7734	0.1702	12.7237	0.0119
	1.5	0.7639	0.1796	24.3270	0.0199	0.7720	0.2291	17.3929	0.0140	0.7718	0.3135	6.8645	0.0128
	2.0	0.7695	0.1756	20.0173	0.0142	0.7748	0.2377	13.0587	0.0127	0.7755	0.3593	4.8870	0.0123
	2.5	0.7611	0.2488	12.6140	0.0167	0.7683	0.4322	9.1347	0.0126	0.7676	0.4632	2.5967	0.0152
4.5	1.0	0.7658	0.0769	56.2960	0.0245	0.7680	0.0942	34.9724	0.0205	0.7695	0.2082	12.7303	0.0135
	1.5	0.7572	0.1626	24.8236	0.0222	0.7687	0.2076	16.7793	0.0162	0.7700	0.3951	6.9856	0.0119
	2.0	0.7597	0.2143	18.2700	0.0166	0.7761	0.2721	11.9715	0.0146	0.7762	0.4437	4.7429	0.0118
	2.5	0.7639	0.2817	11.6547	0.0178	0.7718	0.4179	8.0949	0.0123	0.7713	0.6081	2.9965	0.0113
5.0	1.0	0.7721	0.0771	51.4209	0.0302	0.7688	0.1097	32.2218	0.0237	0.7726	0.2396	12.0238	0.0144
	1.5	0.7669	0.1902	24.3551	0.0255	0.7756	0.2300	16.0382	0.0193	0.7759	0.5548	7.2810	0.0163
	2.0	0.7678	0.2040	16.1194	0.0205	0.7761	0.3224	11.2454	0.0157	0.7782	0.4751	4.1935	0.0126
	2.5	0.7689	0.3327	11.2046	0.0182	0.7764	0.4504	7.6646	0.0125	0.7765	0.6979	2.9451	0.0100
5.5	1.0	0.7881	0.0629	38.9089	0.0306	0.7762	0.1264	27.7289	0.0201	0.7804	0.2188	9.5878	0.0112
	1.5	0.7713	0.1699	20.4970	0.0349	0.7765	0.2475	14.6758	0.0255	0.7771	0.5085	6.2391	0.0149
	2.0	0.7702	0.2527	15.3592	0.0256	0.7791	0.3106	9.8576	0.0184	0.7763	0.6422	4.3709	0.0145
	2.5	0.7677	0.4137	10.6330	0.0162	0.7774	0.4377	6.7929	0.0139	0.7783	0.8612	3.0761	0.0098
6.0	1.0	0.7791	0.0787	37.5289	0.0244	0.7783	0.1302	25.1881	0.0223	0.7815	0.2239	8.7513	0.0148
	1.5	0.7716	0.1903	20.1842	0.0277	0.7780	0.2293	13.2999	0.0216	0.7804	0.3813	4.7546	0.0145
	2.0	0.7696	0.2547	13.0671	0.0216	0.7773	0.3315	8.5217	0.0188	0.7780	0.7958	4.1687	0.0134
	2.5	0.7755	0.3454	9.7694	0.0261	0.7786	0.5518	7.0183	0.0206	0.7824	0.6626	2.3660	0.0157
7.0	1.0	0.7718	0.1264	29.3102	0.0464	0.7797	0.1806	21.8751	0.0377	0.7847	0.1835	6.4737	0.0222
	1.5	0.7697	0.1925	18.3595	0.0301	0.7746	0.3237	13.3570	0.0193	0.7809	0.4868	5.2099	0.0121
	2.0	0.7670	0.2848	11.6166	0.0211	0.7744	0.4615	8.5688	0.0144	0.7791	0.6372	3.1727	0.0114
	2.5	0.7683	0.3362	7.6343	0.0231	0.7742	0.4920	5.2351	0.0161	0.7791	0.8837	2.2422	0.0174

Table 2-2 (f) The fitted parameters (Ep. 1) at normal Maillard reaction of 30°C

Reactant concentration		Red				Green				Blue			
Xylose	Glycine	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.7297	0.0974	87.9961	0.0390	0.7902	0.0691	68.1704	0.0433	0.7586	0.1360	21.1810	0.0164
	1.5	0.7442	0.1245	64.7823	0.0427	0.7681	0.1138	43.9222	0.0311	0.7723	0.2320	13.9389	0.0165
	2.0	0.7250	0.1468	48.0454	0.0398	0.7768	0.1368	31.8077	0.0309	0.7713	0.3243	9.7004	0.0159
	2.5	0.7713	0.1195	35.0383	0.0399	0.7742	0.1849	22.5552	0.0241	0.7738	0.4045	7.2940	0.0171
1.5	1.0	0.7258	0.1094	77.1189	0.0348	0.7764	0.0841	56.1791	0.0371	0.7698	0.1850	17.5535	0.0130
	1.5	0.7661	0.1099	50.8051	0.0401	0.7837	0.1042	33.1068	0.0395	0.7747	0.2684	10.4548	0.0147
	2.0	0.7742	0.1277	31.4384	0.0311	0.7736	0.2017	20.5035	0.0228	0.7756	0.3949	6.6947	0.0114
	2.5	0.7716	0.1635	24.2310	0.0311	0.7783	0.1983	14.2665	0.0231	0.7727	0.4315	4.9931	0.0144
2.0	1.0	0.7491	0.1033	72.0833	0.0403	0.7850	0.0752	49.3977	0.0351	0.7763	0.1930	16.3730	0.0153
	1.5	0.7592	0.0949	46.5830	0.0317	0.7613	0.1212	29.4704	0.0223	0.7661	0.2532	9.0993	0.0168
	2.0	0.7677	0.1330	30.8067	0.0302	0.7709	0.1820	19.1455	0.0211	0.7708	0.4237	6.6777	0.0110
	2.5	0.7533	0.1842	22.0213	0.0271	0.7699	0.2635	14.1394	0.0155	0.7701	0.5480	5.3377	0.0132
2.5	1.0	0.7752	0.0979	53.4169	0.0436	0.7801	0.1064	36.3110	0.0394	0.7764	0.2457	12.4670	0.0123
	1.5	0.7542	0.1178	30.7425	0.0274	0.7587	0.1845	19.7940	0.0199	0.7600	0.3280	6.3926	0.0141
	2.0	0.7693	0.1982	20.0237	0.0220	0.7742	0.2523	12.5622	0.0208	0.7725	0.4302	4.1016	0.0207
	2.5	0.7598	0.2829	15.5090	0.0254	0.7724	0.3485	9.7175	0.0149	0.7667	0.6013	3.4760	0.0138
3.0	1.0	0.8372	0.0746	49.6836	0.0540	0.7793	0.1158	31.6538	0.0352	0.7750	0.2741	10.7495	0.0128
	1.5	0.7575	0.1340	26.0448	0.0303	0.7649	0.2129	17.2726	0.0152	0.7642	0.3520	5.5402	0.0149
	2.0	0.7627	0.2240	14.9848	0.0174	0.7774	0.3053	9.4763	0.0105	0.7801	0.4439	3.2571	0.0149
	2.5	0.7594	0.2913	13.3568	0.0222	0.7673	0.3459	7.5558	0.0188	0.7611	0.7576	3.2232	0.0144
3.5	1.0	0.7738	0.1160	43.4713	0.0557	0.7782	0.1333	28.4260	0.0385	0.7843	0.1032	0.3439	0.0527
	1.5	0.7845	0.1265	23.5710	0.0528	0.7679	0.2313	15.3584	0.0167	0.7655	0.3370	4.4358	0.0189
	2.0	0.7671	0.2871	15.2393	0.0219	0.7824	0.2868	8.8556	0.0134	0.7750	0.6270	3.4928	0.0098
	2.5	0.7650	0.2672	9.3024	0.0233	0.7674	0.4610	6.5161	0.0207	0.7664	0.5024	1.6583	0.0186
4.0	1.0	0.7842	0.1036	37.3393	0.0488	0.7761	0.1511	25.3445	0.0396	0.7741	0.2633	7.3548	0.0201
	1.5	0.7372	0.2275	20.2679	0.0337	0.7740	0.2689	13.6198	0.0174	0.7690	0.4226	4.3135	0.0161
	2.0	0.7736	0.2991	13.9538	0.0229	0.7777	0.3628	8.1938	0.0124	0.7755	0.6394	3.2395	0.0154
	2.5	0.7681	0.3434	9.4121	0.0172	0.7721	0.5150	6.2224	0.0137	0.7691	0.6122	1.9240	0.0151
4.5	1.0	0.7676	0.1421	35.8341	0.0327	0.7778	0.1642	23.1789	0.0263	0.7735	0.3655	8.1634	0.0179
	1.5	0.7514	0.2511	18.6430	0.0274	0.7785	0.3029	12.5537	0.0182	0.7742	0.4077	3.8683	0.0198
	2.0	0.7725	0.3430	12.8081	0.0149	0.7747	0.5177	8.3262	0.0135	0.7731	0.9399	3.5154	0.0081
	2.5	0.7769	0.3286	8.2796	0.0374	0.7778	0.5001	5.4879	0.0229	0.7719	0.8265	1.9700	0.0161
5.0	1.0	0.7684	0.1333	31.8451	0.0215	0.7707	0.1895	21.1060	0.0228	0.7726	0.3934	7.4078	0.0165
	1.5	0.7663	0.2388	16.6028	0.0346	0.7787	0.3699	11.7705	0.0282	0.7734	0.5524	4.0426	0.0265
	2.0	0.7738	0.3842	11.5185	0.0197	0.7760	0.6362	7.9314	0.0128	0.7766	0.9964	3.1874	0.0111
	2.5	0.7760	0.4469	7.8559	0.0376	0.7781	0.7477	5.7427	0.0244	0.7741	1.3326	2.2930	0.0140
5.5	1.0	0.7728	0.1685	23.3910	0.0241	0.7778	0.2573	16.3895	0.0177	0.7805	0.4119	6.6905	0.0132
	1.5	0.7653	0.2622	15.1211	0.0314	0.7796	0.3908	10.7495	0.0246	0.7760	0.5706	3.6802	0.0182
	2.0	0.7758	0.4389	10.6465	0.0197	0.7789	0.6918	7.4590	0.0133	0.7789	1.0426	3.0394	0.0113
	2.5	0.7766	0.4620	6.8678	0.0348	0.7781	0.7372	4.8564	0.0219	0.7747	1.6294	2.1579	0.0108
6.0	1.0	0.7712	0.1714	20.4299	0.0292	0.7770	0.2787	15.1313	0.0293	0.7799	0.3437	5.3026	0.0220
	1.5	0.7794	0.2655	13.6580	0.0386	0.7838	0.3612	9.0045	0.0311	0.7797	0.7193	3.6158	0.0179
	2.0	0.7752	0.4145	9.0018	0.0212	0.7822	0.5754	6.4602	0.0276	0.7809	1.0543	2.7373	0.0094
	2.5	0.7789	0.4613	6.1907	0.0340	0.7808	0.7456	4.5786	0.0253	0.7791	1.0783	1.5950	0.0222
7.0	1.0	0.7900	0.1474	23.7431	0.0436	0.7843	0.2203	15.8040	0.0402	0.7761	0.3847	5.0100	0.0218
	1.5	0.7688	0.3284	12.7610	0.0390	0.7763	0.5149	9.1335	0.0281	0.7785	0.9130	3.5492	0.0179
	2.0	0.7706	0.4898	8.4855	0.0221	0.7757	0.7977	6.0956	0.0140	0.7795	1.3953	2.6729	0.0123
	2.5	0.7687	0.7796	6.4003	0.0251	0.7752	1.0518	4.5416	0.0109	0.7782	1.6413	1.7104	0.0102

Table 2-2 (g) The fitted parameters (Ep. 1) at -0.5℃

Reactant concentration			Red				Green				Blue			
Xylose	Glycine	Na_2HPO_4	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.1	-	-	-	-	0.7392	0.0026	1655.2064	0.0326	0.7419	0.0039	1459.4316	0.0228
	1.5		0.3592	0.0081	961.8010	0.0572	0.7705	0.0037	1346.3460	0.0250	0.7449	0.0047	1212.9709	0.0244
	2.0		0.4157	0.0046	1004.3440	0.0750	0.7783	0.0037	1211.0310	0.0166	0.7475	0.0052	1083.7617	0.0213
	2.5		0.5812	0.0007	2456.7278	0.0803	0.7783	0.0046	1167.2549	0.0208	0.7476	0.0052	1016.9311	0.0248
2.0	1.0		0.5148	0.0071	770.7880	0.0653	0.7782	0.0040	1056.6729	0.0195	0.7592	0.0058	1023.9014	0.0202
	1.5		0.6075	0.0056	713.1825	0.0586	0.7779	0.0051	826.5693	0.0155	0.7630	0.0066	825.3724	0.0230
	2.0		0.6413	0.0031	870.2162	0.0740	0.7809	0.0060	797.1388	0.0140	0.7659	0.0075	759.9427	0.0168
	2.5		0.6835	0.0021	935.2231	0.0633	0.7763	0.0064	719.3604	0.0155	0.7617	0.0081	683.4994	0.0183
3.0	1.0		0.5513	0.0062	789.1774	0.0965	0.7734	0.0055	950.1073	0.0259	0.7602	0.0065	892.1836	0.0159
	1.5		0.6454	0.0042	758.2549	0.0794	0.7726	0.0068	743.8173	0.0182	0.7623	0.0071	693.3189	0.0162
	2.0		0.7233	0.0015	1382.8346	0.0718	0.7770	0.0085	749.6274	0.0202	0.7640	0.0090	680.3029	0.0150
	2.5		0.7203	0.0020	1076.5511	0.0641	0.7710	0.0091	661.0585	0.0169	0.7600	0.0089	585.6176	0.0157
4.0	1.0		0.7442	0.0007	1768.0857	0.0807	0.7737	0.0048	878.1335	0.0226	0.7617	0.0060	783.0564	0.0178
	1.5		0.7155	0.0014	1028.7645	0.0693	0.7714	0.0066	684.7836	0.0166	0.7631	0.0068	611.3392	0.0204
	2.0		0.7486	0.0015	1309.8071	0.0534	0.7761	0.0080	682.7859	0.0180	0.7657	0.0081	587.4677	0.0188
	2.5		0.7418	0.0021	1107.3577	0.0527	0.7720	0.0094	620.0676	0.0178	0.7634	0.0071	485.3251	0.0197
5.0	1.0		0.7581	0.0008	1504.6855	0.0714	0.7774	0.0041	766.9958	0.0256	0.7651	0.0067	711.4972	0.0251
	1.5		0.7366	0.0016	919.3566	0.0638	0.7763	0.0069	627.6151	0.0187	0.7708	0.0073	540.6994	0.0200
	2.0		0.7532	0.0019	1060.7746	0.0517	0.7762	0.0084	615.7007	0.0189	0.7698	0.0083	510.2157	0.0207
	2.5		0.7519	0.0024	987.5387	0.0465	0.7746	0.0097	575.8152	0.0192	0.7680	0.0086	442.0474	0.0169
6.0	1.0		0.7468	0.0010	1328.3472	0.0738	0.7748	0.0058	756.8342	0.0200	0.7709	0.0062	616.8350	0.0162
	1.5		0.7425	0.0019	944.1416	0.0634	0.7765	0.0079	619.3243	0.0163	0.7751	0.0083	515.2152	0.0119
	2.0		0.7522	0.0025	960.5269	0.0518	0.7752	0.0099	584.2426	0.0147	0.7724	0.0103	471.4537	0.0105
	2.5		0.7542	0.0032	908.1526	0.0476	0.7755	0.0112	556.7644	0.0172	0.7735	0.0103	421.7765	0.0101

Table 2-2 (h) The fitted parameters (Ep. 1) at fast reaction of 0°C

Reactant concentration			Red				Green				Blue			
Xylose	Glycine	Na_2HPO_4	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.2	0.4634	0.0040	866.9949	0.0196	0.6438	0.0034	1132.1817	0.0154	0.7614	0.0038	1081.8533	0.0141
		0.3	0.5326	0.0043	684.3034	0.0192	0.6833	0.0034	889.4234	0.0157	0.7772	0.0035	802.0874	0.0149
		0.4	0.5378	0.0035	582.9577	0.0181	0.6927	0.0031	791.7180	0.0153	0.7745	0.0035	698.1458	0.0137
	1.5	0.2	0.5707	0.0051	538.7579	0.0196	0.7095	0.0040	702.7796	0.0149	0.7710	0.0038	657.1471	0.0133
		0.3	0.5759	0.0055	493.7328	0.0205	0.7259	0.0036	610.3937	0.0179	0.7675	0.0043	576.1694	0.0148
		0.4	0.6022	0.0053	453.9569	0.0188	0.7352	0.0041	566.0574	0.0152	0.7753	0.0043	495.5155	0.0146
	2.0	0.2	0.5929	0.0059	475.7623	0.0225	0.7353	0.0043	593.5710	0.0161	0.7669	0.0047	572.0363	0.0130
		0.3	0.6018	0.0076	534.8237	0.0180	0.7464	0.0057	634.3520	0.0168	0.7724	0.0058	563.1273	0.0132
		0.4	0.6467	0.0070	501.0574	0.0230	0.7698	0.0056	559.3273	0.0200	0.7820	0.0061	485.4049	0.0167
1.5	1.0	0.2	0.6076	0.0056	645.9290	0.0195	0.7353	0.0040	808.5274	0.0178	0.7771	0.0045	787.6146	0.0171
		0.3	0.6156	0.0064	600.7011	0.0196	0.7336	0.0053	776.6590	0.0187	0.7743	0.0054	718.9052	0.0168
		0.4	0.6506	0.0064	517.9504	0.0182	0.7479	0.0053	648.0126	0.0162	0.7793	0.0048	555.4647	0.0153
	1.5	0.2	0.6476	0.0081	487.7281	0.0201	0.7524	0.0049	550.8597	0.0211	0.7674	0.0054	552.2985	0.0179
		0.3	0.6696	0.0084	442.7378	0.0190	0.7629	0.0050	476.5228	0.0232	0.7740	0.0055	464.3010	0.0181
		0.4	0.6732	0.0090	405.5330	0.0192	0.7583	0.0053	431.4832	0.0216	0.7650	0.0068	434.7460	0.0173
	2.0	0.2	0.6705	0.0085	414.8358	0.0187	0.7651	0.0053	441.5004	0.0215	0.7723	0.0059	446.3259	0.0168
		0.3	0.7074	0.0099	383.9335	0.0163	0.7742	0.0073	427.7799	0.0168	0.7785	0.0084	428.1144	0.0158
		0.4	0.7244	0.0090	357.1561	0.0206	0.7815	0.0085	425.1354	0.0175	0.7861	0.0075	371.3537	0.0196
2.0	1.0	0.2	0.6391	0.0068	577.0308	0.0228	0.7415	0.0059	733.2820	0.0221	0.7713	0.0060	700.5174	0.0181
		0.3	0.6619	0.0087	527.0500	0.0175	0.7534	0.0058	625.2491	0.0145	0.7765	0.0066	608.8025	0.0128
		0.4	0.6745	0.0071	413.5848	0.0177	0.7549	0.0054	521.7036	0.0134	0.7780	0.0062	514.8500	0.0110
	1.5	0.2	0.7013	0.0080	339.3014	0.0174	0.7722	0.0060	412.5409	0.0135	0.7872	0.0053	381.6601	0.0148
		0.3	0.6967	0.0093	336.9961	0.0190	0.7709	0.0062	396.8703	0.0151	0.7805	0.0065	399.3849	0.0128
		0.4	0.7313	0.0099	285.2890	0.0147	0.7812	0.0059	287.2349	0.0152	0.7889	0.0054	253.0047	0.0165
	2.0	0.2	0.7026	0.0087	318.9290	0.0136	0.7764	0.0063	367.2964	0.0133	0.7836	0.0054	321.6730	0.0141
		0.3	0.7270	0.0121	317.5727	0.0121	0.7785	0.0089	357.5872	0.0137	0.7822	0.0084	341.9841	0.0108
		0.4	0.7469	0.0146	302.7532	0.0133	0.7839	0.0104	329.2602	0.0136	0.7855	0.0113	325.4385	0.0118
2.5	1.0	0.2	0.6331	0.0081	535.8071	0.0214	0.7450	0.0064	682.9248	0.0198	0.7682	0.0069	660.2187	0.0178
		0.3	0.7013	0.0075	349.3218	0.0199	0.7636	0.0058	456.6406	0.0168	0.7817	0.0052	425.4764	0.0192
		0.4	0.7028	0.0089	375.2098	0.0201	0.7638	0.0063	456.2721	0.0188	0.7786	0.0060	417.8602	0.0173
	1.5	0.2	0.6877	0.0090	350.4145	0.0188	0.7661	0.0054	383.7610	0.0188	0.7731	0.0057	389.9034	0.0171
		0.3	0.7136	0.0101	273.3493	0.0206	0.7693	0.0061	296.2431	0.0187	0.7711	0.0058	281.8035	0.0183
		0.4	0.7201	0.0121	270.9616	0.0168	0.7666	0.0076	302.1722	0.0182	0.7698	0.0068	271.2079	0.0153
	2.0	0.2	0.6988	0.0097	301.5605	0.0157	0.7734	0.0069	340.2633	0.0162	0.7745	0.0072	340.4388	0.0139
		0.3	0.7336	0.0138	282.7717	0.0147	0.7748	0.0106	323.6261	0.0149	0.7775	0.0099	314.1579	0.0138
		0.4	0.7537	0.0148	255.6587	0.0183	0.7808	0.0129	296.2761	0.0144	0.7818	0.0121	286.0655	0.0130

Table 2-2 (i) The fitted parameters (Ep. 1) at fast reaction of 5°C

Reactant concentration			Red				Green				Blue			
Xylose	Glycine	Na_2HPO_4	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.2	0.4941	0.0127	243.0964	0.0235	0.6819	0.0060	329.9472	0.0232	0.7607	0.0068	335.1708	0.0168
		0.3	0.5314	0.0111	217.1586	0.0224	0.7100	0.0064	305.2161	0.0223	0.7671	0.0068	273.3710	0.0179
		0.4	0.5286	0.0102	205.6395	0.0280	0.7254	0.0054	270.4912	0.0251	0.7651	0.0088	278.0963	0.0189
	1.5	0.2	0.6242	0.0128	211.5125	0.0182	0.7552	0.0102	261.3598	0.0156	0.7823	0.0116	260.3671	0.0134
		0.3	0.6508	0.0112	182.9002	0.0197	0.7684	0.0108	246.3681	0.0146	0.7844	0.0131	243.5026	0.0147
		0.4	0.6481	0.0099	186.6289	0.0214	0.7654	0.0115	256.7826	0.0167	0.7782	0.0125	228.3292	0.0194
	2.0	0.2	0.6099	0.0109	170.9390	0.0230	0.7530	0.0101	232.1800	0.0173	0.7709	0.0107	216.4497	0.0148
		0.3	0.6629	0.0093	131.5546	0.0203	0.7686	0.0106	196.5043	0.0162	0.7744	0.0118	182.2135	0.0170
		0.4	0.6775	0.0111	147.2109	0.0247	0.7779	0.0084	151.7965	0.0213	0.7786	0.0114	154.0142	0.0180
1.5	1.0	0.2	0.5985	0.0180	198.2604	0.0241	0.7419	0.0111	267.9100	0.0238	0.7710	0.0103	243.2826	0.0165
		0.3	0.6364	0.0201	191.0109	0.0240	0.7517	0.0110	226.0983	0.0218	0.7682	0.0121	215.8257	0.0170
		0.4	0.6261	0.0202	194.0911	0.0252	0.7490	0.0105	218.5819	0.0246	0.7632	0.0115	202.3933	0.0211
	1.5	0.2	0.6461	0.0170	163.3540	0.0238	0.7649	0.0115	201.3174	0.0195	0.7736	0.0121	193.3095	0.0161
		0.3	0.6831	0.0175	148.2311	0.0177	0.7707	0.0109	168.8395	0.0139	0.7768	0.0131	182.7833	0.0139
		0.4	0.6993	0.0170	127.0765	0.0180	0.7730	0.0119	154.9566	0.0121	0.7792	0.0105	119.9339	0.0170
	2.0	0.2	0.6595	0.0214	146.7722	0.0251	0.7682	0.0124	156.8831	0.0202	0.7716	0.0133	156.8420	0.0165
		0.3	0.7067	0.0282	144.1511	0.0221	0.7659	0.0265	170.5216	0.0218	0.7690	0.0232	154.4378	0.0161
		0.4	0.7137	0.0287	134.8982	0.0203	0.7608	0.0228	145.0785	0.0170	0.7633	0.0237	138.6131	0.0156
2.0	1.0	0.2	0.6253	0.0246	183.3830	0.0286	0.7475	0.0121	207.7657	0.0231	0.7653	0.0115	198.2910	0.0169
		0.3	0.6569	0.0177	144.0808	0.0228	0.7649	0.0088	160.7099	0.0193	0.7717	0.0123	188.6412	0.0158
		0.4	0.6860	0.0200	142.9688	0.0223	0.7665	0.0145	181.8280	0.0195	0.7745	0.0160	181.5255	0.0163
	1.5	0.2	0.6703	0.0195	145.4334	0.0186	0.7677	0.0124	165.2933	0.0142	0.7755	0.0109	150.4541	0.0194
		0.3	0.7045	0.0231	119.6138	0.0199	0.7717	0.0138	132.5913	0.0162	0.7759	0.0124	123.1392	0.0167
		0.4	0.7272	0.0216	110.3619	0.0159	0.7707	0.0164	128.2957	0.0145	0.7731	0.0175	127.9853	0.0130
	2.0	0.2	0.6875	0.0201	128.9213	0.0262	0.7603	0.0184	147.5784	0.0178	0.7610	0.0165	131.5654	0.0176
		0.3	0.7131	0.0282	110.2270	0.0184	0.7608	0.0227	122.8579	0.0137	0.7608	0.0209	120.7228	0.0178
		0.4	0.7371	0.0330	105.7903	0.0157	0.7649	0.0240	107.1808	0.0117	0.7663	0.0223	98.8728	0.0134
2.5	1.0	0.2	0.6579	0.0245	162.6130	0.0221	0.7681	0.0118	181.8285	0.0195	0.7757	0.0134	191.0768	0.0151
		0.3	0.7073	0.0219	122.7375	0.0193	0.7764	0.0148	155.9945	0.0175	0.7837	0.0162	166.8684	0.0165
		0.4	0.7217	0.0243	113.8374	0.0173	0.7754	0.0144	123.9649	0.0187	0.7853	0.0136	110.0284	0.0163
	1.5	0.2	0.7007	0.0267	130.8986	0.0198	0.7719	0.0176	143.3444	0.0151	0.7803	0.0122	108.7594	0.0146
		0.3	0.7231	0.0237	101.2667	0.0190	0.7761	0.0169	122.0662	0.0161	0.7828	0.0140	109.3071	0.0142
		0.4	0.7466	0.0342	104.6469	0.0153	0.7761	0.0200	102.9129	0.0122	0.7705	0.0194	116.3832	0.0130
	2.0	0.2	0.7166	0.0238	110.7068	0.0198	0.7698	0.0184	116.4049	0.0130	0.7784	0.0282	107.7412	0.0115
		0.3	0.7441	0.0331	90.4569	0.0153	0.7744	0.0293	105.8400	0.0107	0.7818	0.0289	79.9418	0.0092
		0.4	0.7580	0.0354	72.8238	0.0146	0.7738	0.0292	80.8831	0.0108	0.7607	0.0068	335.1708	0.0168

Table 2-2 (j) The fitted parameters (Ep. 1) at fast reaction of 10°C

Reactant concentration			Red				Green				Blue			
Xylose	Glycine	Na_2HPO_4	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.2	0.6084	0.0181	123.8143	0.0161	0.7306	0.0115	141.4427	0.0148	0.7917	0.0100	125.7707	0.0204
		0.3	0.6282	0.0213	121.8804	0.0169	0.7409	0.0157	148.7797	0.0188	0.7767	0.0156	131.7251	0.0131
		0.4	0.6031	0.0247	137.7211	0.0203	0.7450	0.0136	150.6331	0.0197	0.7799	0.0156	136.1829	0.0154
	1.5	0.2	0.6655	0.0228	109.9505	0.0119	0.7640	0.0180	125.1263	0.0145	0.7859	0.0172	112.8593	0.0116
		0.3	0.6766	0.0247	108.5113	0.0119	0.7664	0.0197	122.7213	0.0146	0.7810	0.0208	115.3214	0.0107
		0.4	0.6972	0.0286	111.3824	0.0185	0.7759	0.0239	120.4338	0.0175	0.7851	0.0223	99.1215	0.0123
	2.0	0.2	0.6699	0.0227	97.6774	0.0180	0.7634	0.0189	110.0923	0.0168	0.7758	0.0178	94.0895	0.0153
		0.3	0.7112	0.0299	98.8558	0.0201	0.7775	0.0230	99.3256	0.0190	0.7808	0.0232	88.5914	0.0147
		0.4	0.7242	0.0319	101.9608	0.0227	0.7763	0.0263	97.6248	0.0166	0.7791	0.0267	83.8348	0.0136
1.5	1.0	0.2	0.6356	0.0239	120.9017	0.0209	0.7501	0.0194	157.5346	0.0217	0.7709	0.0218	152.1555	0.0191
		0.3	0.6568	0.0319	111.6022	0.0177	0.7519	0.0186	119.6693	0.0234	0.7626	0.0224	118.3662	0.0178
		0.4	0.6895	0.0324	106.6224	0.0197	0.7607	0.0273	126.3429	0.0205	0.7745	0.0223	102.0896	0.0158
	1.5	0.2	0.6963	0.0336	97.6722	0.0159	0.7705	0.0294	114.6458	0.0164	0.7775	0.0347	116.9632	0.0123
		0.3	0.7323	0.0456	88.0419	0.0145	0.7781	0.0294	87.9000	0.0160	0.7847	0.0290	82.2515	0.0125
		0.4	0.7349	0.0462	84.8860	0.0158	0.7713	0.0359	89.2236	0.0136	0.7786	0.0302	74.2815	0.0118
	2.0	0.2	0.7220	0.0386	87.4981	0.0144	0.7739	0.0278	85.7088	0.0175	0.7765	0.0314	88.2961	0.0148
		0.3	0.7255	0.0478	76.8529	0.0165	0.7608	0.0373	80.0740	0.0172	0.7629	0.0359	75.5560	0.0173
		0.4	0.7465	0.0514	71.9975	0.0150	0.7702	0.0407	72.4891	0.0148	0.7740	0.0431	72.5083	0.0122
2.0	1.0	0.2	0.6711	0.0325	92.9121	0.0135	0.7578	0.0182	98.2849	0.0187	0.7700	0.0208	105.8627	0.0170
		0.3	0.6998	0.0357	85.1290	0.0156	0.7664	0.0221	91.9033	0.0195	0.7715	0.0256	93.3844	0.0158
		0.4	0.7191	0.0419	87.0842	0.0175	0.7676	0.0306	97.3367	0.0176	0.7761	0.0265	84.5226	0.0161
	1.5	0.2	0.7234	0.0387	80.2599	0.0124	0.7764	0.0311	86.5872	0.0135	0.7813	0.0306	83.3303	0.0112
		0.3	0.7458	0.0549	73.7779	0.0131	0.7756	0.0410	76.8872	0.0124	0.7793	0.0382	75.0732	0.0118
		0.4	0.7594	0.0557	69.7021	0.0135	0.7808	0.0432	71.8458	0.0129	0.7848	0.0329	59.0094	0.0158
	2.0	0.2	0.7231	0.0412	71.9177	0.0168	0.7672	0.0338	73.9054	0.0168	0.7698	0.0324	70.0965	0.0157
		0.3	0.7538	0.0617	65.7329	0.0144	0.7738	0.0476	66.4874	0.0126	0.7759	0.0510	68.7772	0.0119
		0.4	0.7557	0.0647	58.0373	0.0121	0.7685	0.0622	64.9764	0.0109	0.7727	0.0522	60.3601	0.0121
2.5	1.0	0.2	0.6901	0.0326	86.5614	0.0167	0.7669	0.0268	107.1372	0.0193	0.7776	0.0247	100.9958	0.0173
		0.3	0.7327	0.0448	76.5861	0.0168	0.7791	0.0315	85.3688	0.0219	0.7860	0.0305	83.3346	0.0180
		0.4	0.7406	0.0500	72.4114	0.0151	0.7776	0.0301	73.0325	0.0180	0.7854	0.0297	67.8700	0.0159
	1.5	0.2	0.7326	0.0412	72.2618	0.0178	0.7771	0.0376	83.3487	0.0143	0.7797	0.0438	89.0612	0.0120
		0.3	0.7590	0.0657	63.7741	0.0142	0.7810	0.0402	59.8712	0.0192	0.7849	0.0432	66.2639	0.0151
		0.4	0.7651	0.0714	59.8533	0.0128	0.7786	0.0528	61.3727	0.0136	0.7866	0.0460	58.4489	0.0142
	2.0	0.2	0.7497	0.0506	70.1632	0.0179	0.7780	0.0521	74.8254	0.0109	0.7805	0.0471	71.6491	0.0120
		0.3	0.7706	0.0566	55.4944	0.0197	0.7794	0.0691	63.0505	0.0123	0.7851	0.0558	59.0827	0.0112
		0.4	0.7744	0.0715	48.8218	0.0114	0.7810	0.0525	48.7635	0.0139	0.7887	0.0573	52.5929	0.0096

Table 2-2 (k) The fitted parameters (Ep. 1) at fast reaction of 15℃

Reactant concentration			Red				Green				Blue			
Xylose	Glycine	Na_2HPO_4	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.2	0.6294	0.0237	61.6370	0.0231	0.7508	0.0222	78.9779	0.0166	0.7673	0.0314	79.3556	0.0128
		0.3	0.6840	0.0287	68.1534	0.0280	0.7699	0.0232	64.8973	0.0157	0.7767	0.0244	46.1884	0.0141
		0.4	0.7129	0.0300	70.3867	0.0257	0.7718	0.0297	66.2046	0.0144	0.7754	0.0348	55.5759	0.0132
	1.5	0.2	0.7069	0.0353	56.5745	0.0239	0.7806	0.0296	54.7544	0.0164	0.7847	0.0344	52.5073	0.0145
		0.3	0.7379	0.0416	56.1547	0.0233	0.7751	0.0452	58.0558	0.0142	0.7789	0.0416	45.5154	0.0134
		0.4	0.7501	0.0509	60.3123	0.0229	0.7795	0.0470	54.8370	0.0177	0.7800	0.0545	47.2951	0.0120
	2.0	0.2	0.7208	0.0327	48.4930	0.0245	0.7672	0.0368	49.4615	0.0192	0.7682	0.0417	45.6197	0.0159
		0.3	0.7523	0.0450	45.2980	0.0219	0.7735	0.0513	45.6493	0.0161	0.7762	0.0443	33.8320	0.0154
		0.4	0.7559	0.0643	47.7282	0.0173	0.7705	0.0561	40.2560	0.0164	0.7726	0.0611	35.1134	0.0118
1.5	1.0	0.2	0.7084	0.0429	66.1029	0.0284	0.7744	0.0362	69.6424	0.0187	0.7809	0.0329	52.7200	0.0199
		0.3	0.7238	0.0427	59.8497	0.0236	0.7661	0.0470	66.1754	0.0166	0.7683	0.0464	55.5712	0.0151
		0.4	0.7412	0.0517	53.9244	0.0233	0.7692	0.0543	58.3569	0.0160	0.7732	0.0511	47.3864	0.0147
	1.5	0.2	0.7312	0.0592	54.3028	0.0206	0.7747	0.0614	58.8837	0.0122	0.7777	0.0569	51.4011	0.0117
		0.3	0.7566	0.0716	44.2248	0.0163	0.7801	0.0575	41.3392	0.0156	0.7847	0.0489	33.0607	0.0166
		0.4	0.7573	0.0739	41.3691	0.0141	0.7740	0.0762	44.4173	0.0097	0.7781	0.0650	34.1184	0.0125
	2.0	0.2	0.7502	0.0517	45.2520	0.0182	0.7765	0.0583	47.3502	0.0128	0.7785	0.0531	40.1982	0.0163
		0.3	0.7545	0.0814	34.8145	0.0173	0.7667	0.0644	29.6439	0.0242	0.7704	0.0702	29.5591	0.0199
		0.4	0.7592	0.0903	32.9221	0.0165	0.7681	0.0962	35.1267	0.0146	0.7707	0.0728	25.6120	0.0188
2.0	1.0	0.2	0.7252	0.0481	43.4765	0.0239	0.7683	0.0474	50.6022	0.0163	0.7725	0.0444	44.6858	0.0142
		0.3	0.7485	0.0669	43.8183	0.0206	0.7732	0.0516	42.1750	0.0142	0.7770	0.0457	34.8340	0.0157
		0.4	0.7582	0.0682	40.2188	0.0165	0.7770	0.0671	45.0609	0.0109	0.7800	0.0594	36.1677	0.0114
	1.5	0.2	0.7496	0.0606	34.0604	0.0205	0.7763	0.0625	37.6352	0.0143	0.7799	0.0520	32.1028	0.0143
		0.3	0.7552	0.0866	33.8481	0.0140	0.7701	0.0650	30.3270	0.0179	0.7743	0.0705	31.1463	0.0134
		0.4	0.7649	0.0949	30.3283	0.0159	0.7764	0.0919	34.0425	0.0127	0.7782	0.0728	25.7030	0.0172
	2.0	0.2	0.7441	0.0893	35.2964	0.0197	0.7638	0.0676	30.5397	0.0211	0.7653	0.0734	32.0622	0.0188
		0.3	0.7582	0.1018	27.5582	0.0168	0.7672	0.0796	21.2227	0.0206	0.7692	0.0835	21.7597	0.0169
		0.4	0.7605	0.0878	23.3940	0.0198	0.7669	0.1102	25.6869	0.0154	0.7702	0.0808	17.6074	0.0207
2.5	1.0	0.2	0.7536	0.0695	41.8462	0.0211	0.7805	0.0655	46.9640	0.0140	0.7858	0.0580	41.5936	0.0128
		0.3	0.7663	0.0777	32.1052	0.0179	0.7823	0.0718	36.5769	0.0147	0.7877	0.0581	31.1796	0.0139
		0.4	0.7684	0.0894	33.4583	0.0136	0.7818	0.0647	31.4380	0.0144	0.7881	0.0732	31.8500	0.0114
	1.5	0.2	0.7600	0.0904	32.1121	0.0184	0.7783	0.0879	35.3157	0.0137	0.7835	0.0700	31.3384	0.0156
		0.3	0.7695	0.0965	27.3073	0.0140	0.7797	0.0936	31.8775	0.0107	0.7848	0.0758	25.2047	0.0147
		0.4	0.7687	0.0790	23.6567	0.0237	0.7726	0.0932	20.0992	0.0214	0.7808	0.0983	21.2551	0.0137
	2.0	0.2	0.7667	0.0780	29.2041	0.0150	0.7772	0.0936	29.3733	0.0119	0.7812	0.0757	22.6387	0.0173
		0.3	0.7705	0.1343	19.3433	0.0183	0.7775	0.1247	21.9314	0.0164	0.7831	0.0916	16.6915	0.0180
		0.4	0.7673	0.1409	16.5749	0.0187	0.7743	0.1205	19.8304	0.0159	0.7822	0.0854	11.6480	0.0168

Table 2-2 (l) The fitted parameters (Ep. 1) at fast reaction of 20℃

Reactant concentration			Red				Green				Blue			
Xylose	Glycine	Na_2HPO_4	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.2	0.7138	0.0569	49.3226	0.0235	0.7767	0.0549	54.1167	0.0154	0.7821	0.0559	47.0276	0.0082
		0.3	0.7420	0.0699	45.6177	0.0184	0.7823	0.0676	48.3236	0.0133	0.7855	0.0673	40.7550	0.0068
		0.4	0.7609	0.0711	39.0126	0.0170	0.7868	0.0702	40.6675	0.0115	0.7875	0.0695	32.7911	0.0061
	1.5	0.2	0.7551	0.0827	41.1864	0.0189	0.7811	0.0862	42.8141	0.0115	0.7782	0.0884	39.3676	0.0066
		0.3	0.7679	0.1075	36.0083	0.0130	0.7790	0.1071	36.7197	0.0088	0.7748	0.1027	32.1613	0.0065
		0.4	0.7735	0.1225	33.0516	0.0103	0.7804	0.1204	33.4635	0.0075	0.7780	0.1030	27.1862	0.0092
	2.0	0.2	0.7640	0.1000	34.7045	0.0166	0.7765	0.1051	35.3003	0.0101	0.7733	0.0994	31.5549	0.0084
		0.3	0.7752	0.1271	31.5223	0.0098	0.7793	0.1223	31.4287	0.0086	0.7781	0.1131	27.7553	0.0074
		0.4	0.7842	0.1538	27.1724	0.0058	0.7861	0.1490	27.3138	0.0062	0.7860	0.1233	22.3158	0.0078
1.5	1.0	0.2	0.7643	0.0686	37.4153	0.0178	0.7771	0.0760	42.4518	0.0081	0.7785	0.0676	35.1961	0.0079
		0.3	0.7700	0.0908	35.0618	0.0108	0.7765	0.0884	37.4505	0.0073	0.7763	0.0853	32.4795	0.0072
		0.4	0.7620	0.1042	32.4525	0.0104	0.7663	0.1051	34.9767	0.0109	0.7638	0.0964	28.9300	0.0126
	1.5	0.2	0.7574	0.1013	33.2306	0.0190	0.7845	0.0964	35.0861	0.0132	0.7838	0.1021	33.3339	0.0088
		0.3	0.7623	0.1293	30.4966	0.0153	0.7762	0.1332	33.0295	0.0107	0.7748	0.1159	29.1045	0.0058
		0.4	0.7569	0.1521	28.3631	0.0107	0.7659	0.1417	29.5910	0.0075	0.7669	0.1268	25.5381	0.0051
	2.0	0.2	0.7714	0.1130	30.6265	0.0159	0.7852	0.1326	32.8609	0.0109	0.7844	0.1198	29.8328	0.0077
		0.3	0.7781	0.1627	27.2236	0.0124	0.7846	0.1586	28.3180	0.0075	0.7817	0.1394	25.5040	0.0052
		0.4	0.7785	0.2000	24.2952	0.0085	0.7812	0.1885	25.2397	0.0063	0.7794	0.1634	22.2552	0.0065
2.0	1.0	0.2	0.7645	0.0890	36.8189	0.0164	0.7724	0.0892	39.0592	0.0096	0.7708	0.0841	35.0284	0.0088
		0.3	0.7671	0.1280	31.0215	0.0114	0.7709	0.1111	32.1702	0.0115	0.7710	0.0976	27.1722	0.0119
		0.4	0.7731	0.1398	30.4780	0.0083	0.7751	0.1294	32.0800	0.0079	0.7741	0.1414	30.3930	0.0071
	1.5	0.2	0.7483	0.1328	29.5683	0.0155	0.7668	0.1312	31.5187	0.0101	0.7653	0.1161	28.9043	0.0059
		0.3	0.7695	0.1611	24.7549	0.0103	0.7787	0.1446	26.0720	0.0069	0.7785	0.1269	23.0798	0.0054
		0.4	0.7636	0.1913	22.7034	0.0058	0.7681	0.1706	23.8186	0.0048	0.7708	0.1477	20.6621	0.0048
	2.0	0.2	0.7731	0.1583	25.3098	0.0137	0.7814	0.1658	26.4207	0.0097	0.7801	0.1275	22.7654	0.0117
		0.3	0.7784	0.2142	22.0585	0.0099	0.7815	0.1998	22.9183	0.0084	0.7801	0.1553	20.1688	0.0078
		0.4	0.7816	0.2352	19.6608	0.0067	0.7831	0.2125	20.4982	0.0064	0.7826	0.1819	18.2973	0.0062
2.5	1.0	0.2	0.7757	0.1178	31.0509	0.0100	0.7802	0.1101	32.8568	0.0069	0.7803	0.0955	29.7047	0.0059
		0.3	0.7829	0.1485	26.2011	0.0048	0.7841	0.1371	28.1140	0.0050	0.7844	0.1222	25.2955	0.0054
		0.4	0.7844	0.1614	23.6508	0.0053	0.7850	0.1426	25.0288	0.0057	0.7858	0.1255	21.3360	0.0067
	1.5	0.2	0.7702	0.1399	21.3869	0.0099	0.7809	0.1352	23.2740	0.0063	0.7835	0.1050	19.5901	0.0064
		0.3	0.7803	0.1874	19.9068	0.0061	0.7829	0.1717	21.2495	0.0052	0.7829	0.1464	19.1840	0.0047
		0.4	0.7845	0.2012	17.8665	0.0099	0.7859	0.1874	19.3157	0.0089	0.7877	0.1552	16.3200	0.0091
	2.0	0.2	0.7792	0.1867	20.7033	0.0106	0.7849	0.1745	21.4201	0.0076	0.7838	0.1528	19.7337	0.0077
		0.3	0.7852	0.2565	18.0193	0.0053	0.7866	0.2189	18.5993	0.0050	0.7867	0.1802	16.4933	0.0060
		0.4	0.7661	0.2158	13.9151	0.0191	0.7673	0.2119	15.1743	0.0166	0.7727	0.1779	12.2998	0.0152

Table 2-2 (m) The fitted parameters (Ep. 1) at fast reaction of 25°C

Reactant concentration			Red				Green				Blue			
Xylose	Glycine	Na_2HPO_4	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE	ΔC_v	r_{max}	T_{inf}	RMSE
1.0	1.0	0.2	0.7701	0.1048	28.4317	0.0170	0.7830	0.1219	28.7775	0.0070	0.7830	0.1219	24.4785	0.0055
		0.3	0.7786	0.1329	25.8446	0.0120	0.7858	0.1451	25.7733	0.0064	0.7847	0.1379	21.3892	0.0071
		0.4	0.7842	0.1545	20.7365	0.0075	0.7883	0.1686	21.3222	0.0052	0.7884	0.1516	16.2017	0.0068
	1.5	0.2	0.7732	0.1523	22.4158	0.0111	0.7855	0.1668	22.7712	0.0066	0.7884	0.1546	19.3723	0.0076
		0.3	0.7798	0.1915	18.9127	0.0053	0.7862	0.2085	19.7393	0.0060	0.7881	0.1710	15.3787	0.0109
		0.4	0.7803	0.2191	17.9856	0.0051	0.7851	0.2103	17.8063	0.0072	0.7861	0.1854	13.4785	0.0093
	2.0	0.2	0.7726	0.1854	21.1672	0.0087	0.7794	0.2242	21.5680	0.0058	0.7793	0.1824	18.2084	0.0086
		0.3	0.7760	0.2452	17.8954	0.0059	0.7788	0.2530	17.9868	0.0073	0.7790	0.2121	14.6997	0.0094
		0.4	0.7840	0.2778	16.0822	0.0048	0.7856	0.2793	16.1496	0.0059	0.7861	0.2514	13.0479	0.0070
1.5	1.0	0.2	0.7685	0.1494	21.1231	0.0128	0.7845	0.1507	22.2135	0.0071	0.7855	0.1449	19.2573	0.0077
		0.3	0.7646	0.1849	19.5859	0.0103	0.7749	0.1940	20.7328	0.0088	0.7744	0.1861	18.1661	0.0090
		0.4	0.7673	0.2046	17.8647	0.0098	0.7763	0.2212	18.8728	0.0102	0.7796	0.1928	15.3012	0.0108
	1.5	0.2	0.7526	0.2108	15.3882	0.0091	0.7725	0.2191	16.5304	0.0080	0.7869	0.1836	14.2057	0.0104
		0.3	0.7609	0.2733	14.2850	0.0118	0.7728	0.2662	14.7739	0.0127	0.7810	0.2189	12.2027	0.0130
		0.4	0.7568	0.3170	14.1695	0.0109	0.7688	0.3094	14.6508	0.0100	0.7733	0.2784	12.3833	0.0078
	2.0	0.2	0.7563	0.2666	14.9696	0.0100	0.7731	0.2618	15.1598	0.0122	0.7771	0.2408	13.7216	0.0111
		0.3	0.7673	0.3566	13.4248	0.0097	0.7762	0.3454	13.6774	0.0099	0.7773	0.3049	12.1258	0.0094
		0.4	0.7672	0.4182	13.1809	0.0104	0.7743	0.4407	13.6931	0.0118	0.7767	0.3290	11.4651	0.0121
2.0	1.0	0.2	0.7636	0.1809	19.6258	0.0119	0.7754	0.1922	20.7968	0.0092	0.7756	0.1678	18.1781	0.0115
		0.3	0.7718	0.2179	16.6978	0.0101	0.7779	0.2252	17.9060	0.0085	0.7782	0.1886	15.1164	0.0108
		0.4	0.7716	0.2959	15.2743	0.0068	0.7754	0.2738	15.7832	0.0081	0.7760	0.2433	13.4493	0.0087
	1.5	0.2	0.7469	0.2755	14.8551	0.0115	0.7701	0.2689	15.4952	0.0110	0.7748	0.2299	13.8731	0.0116
		0.3	0.7699	0.3668	13.1658	0.0091	0.7806	0.3428	13.7133	0.0080	0.7805	0.3117	12.3832	0.0057
		0.4	0.7769	0.3935	11.0208	0.0059	0.7834	0.3577	11.4132	0.0066	0.7888	0.2995	9.5052	0.0094
	2.0	0.2	0.7630	0.3375	13.1048	0.0087	0.7802	0.3354	13.3414	0.0076	0.7803	0.3011	12.1903	0.0070
		0.3	0.7741	0.4212	11.2537	0.0060	0.7813	0.3914	11.4136	0.0069	0.7817	0.3440	10.2944	0.0094
		0.4	0.7770	0.4849	10.7883	0.0077	0.7816	0.4613	11.1038	0.0080	0.7820	0.3737	9.7126	0.0108
2.5	1.0	0.2	0.7729	0.2288	15.5931	0.0086	0.7798	0.2198	16.5555	0.0082	0.7797	0.2096	15.0919	0.0084
		0.3	0.7817	0.2766	13.3088	0.0060	0.7845	0.2544	14.0723	0.0065	0.7853	0.2237	12.0574	0.0075
		0.4	0.7800	0.3099	11.7110	0.0060	0.7810	0.2770	12.2371	0.0071	0.7858	0.2282	9.5491	0.0092
	1.5	0.2	0.7710	0.3217	11.4520	0.0085	0.7823	0.2813	11.7079	0.0080	0.7875	0.2251	9.9735	0.0115
		0.3	0.7802	0.3840	10.1206	0.0058	0.7861	0.3557	10.7152	0.0060	0.7862	0.2958	9.1869	0.0081
		0.4	0.7818	0.4690	9.3822	0.0062	0.7840	0.3886	9.4242	0.0094	0.7885	0.3263	7.7654	0.0115
	2.0	0.2	0.7735	0.3905	10.8161	0.0073	0.7824	0.3708	11.0090	0.0075	0.7823	0.3286	10.2265	0.0065
		0.3	0.7812	0.4952	9.2747	0.0061	0.7841	0.4695	9.7244	0.0075	0.7841	0.3882	8.5471	0.0076
		0.4	0.7781	0.5980	8.3360	0.0084	0.7786	0.4990	8.4247	0.0104	0.7821	0.4090	7.0199	0.0123

2.3.3. Effect of reactant concentration and temperature on color change

The observed color change kinetics was influenced by reactant concentration and reaction temperature, with representative relationships shown in Fig. 2-7 (a ~ d). At the Fig. 2-7 (a), (b), (c), (d) compared difference of reaction rate by reaction conditions of D-xylose, glycine, Na_2HPO_4, and reaction temperature, respectively. For all conditions, increasing the substrate concentration and temperature resulted in a faster reaction. However, the reaction rate did not significantly change at higher concentrations. In contrast, the reaction rate markedly increased with increasing temperature, with observable color changes detected even at temperatures as low as 5 and 0 ℃. These results that reaction rate is influenced by temperature and concentration conditions allowed flexibility about the reaction completion time. The completion times by reaction shown in the Table 2-3. But the empty space in Table have very long reaction time of over than 1 years, or unexpressed the threshold values.

The completion times at normal reaction were 236–415, 138–424, 60–415, 29–316, 15–242, and 10–121 days at 5, 10, 15, 20, 25 and 30 ℃, respectively. And the completion times at fast reaction were 8–61, 7–21, 4–14, 2–10, 1–4, and 0.5–2 days at 0, 5, 10, 15, 20 and 25 ℃, respectively.

Although a large number of TTIs have been proposed so far, most of the existing ones are limited by narrow temperature ranges, reaction reliability and stability, safety of used materials, etc. In particular, a number of applications cannot be realized due to the fact that most existing TTIs are intended for chilled food distribution. In contrast, the color variation rate of the Maillard reaction described herein allows the reaction time to be adjusted from 12 h to 1 years by controlling reaction temperature and reactant concentrations. Thus, Maillard reaction-based TTIs can potentially not only indicate the shelf life of various types of foods, including those stored chilled and at ambient temperature, but also help to establish optimum ripening times of fruits and/or vegetables. Furthermore, the harvest time of fruits and/or vegetables during cultivation periods can also be optimized based on accumulation temperature.

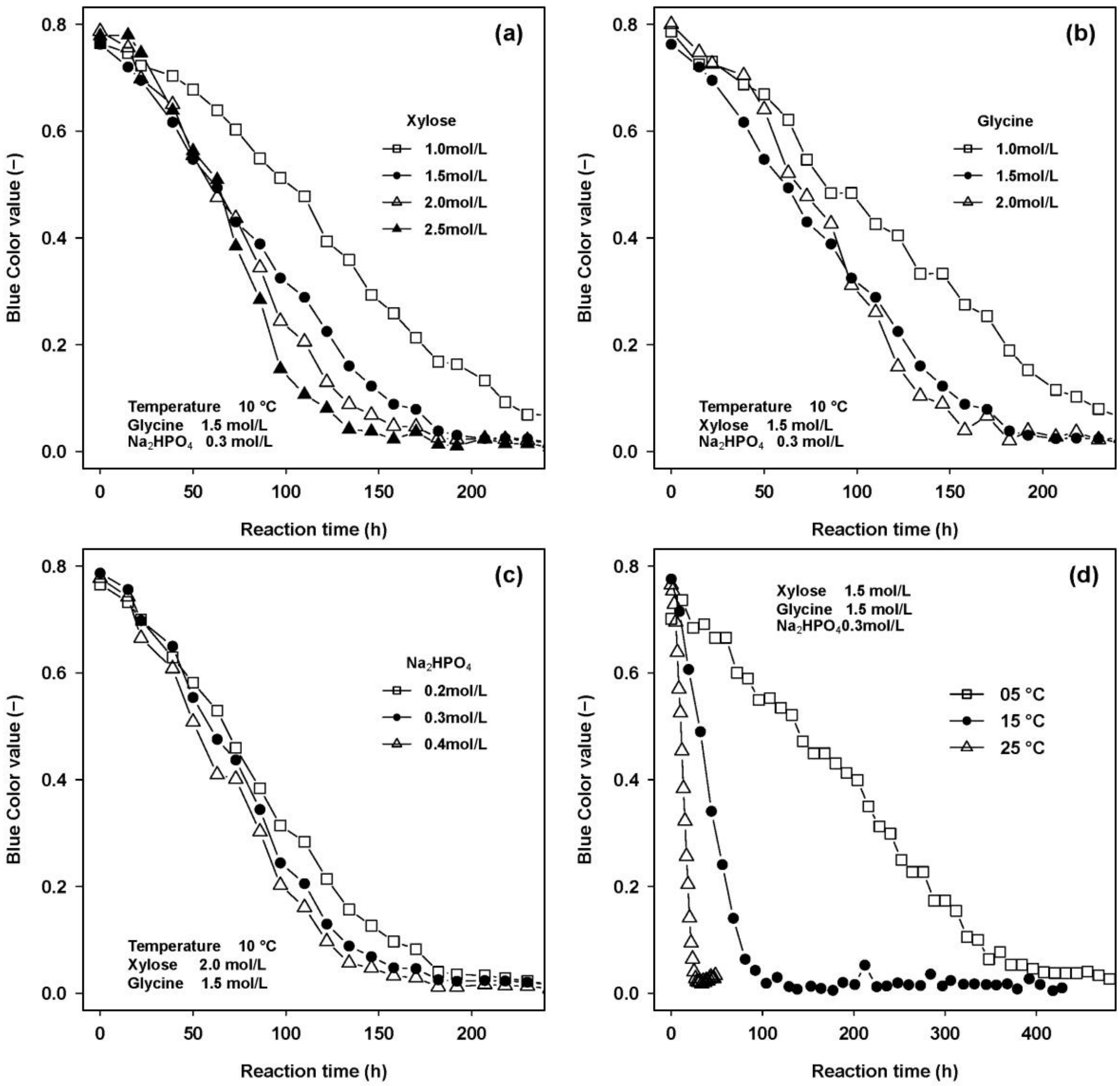


Fig. 2-7 Representative B value variations as a function of reaction time and concentrations of D-xylose (a), glycine (b), Na_2HPO_4 (c), and temperature (d)

Table 2-3(a) The completion times at normal Maillard reaction

Reactant concentration		Completion times (d)					
Xylose	Glycine	5	10	15	20	25	30
1.0	1.0					242	121
	1.5				316	149	87
	2.0			415	233	113	71
	2.5			331	163	82	55
1.5	1.0				406	189	108
	1.5				221	113	73
	2.0			307	159	81	50
	2.5			226	113	59	39
2.0	1.0			585	310	153	98
	1.5			361	170	92	73
	2.0			237	120	63	49
	2.5			171	84	43	37
2.5	1.0			479	259	138	77
	1.5			282	145	76	53
	2.0		424	195	99	52	32
	2.5	562	310	149	72	36	25
3.0	1.0			439	224	117	72
	1.5		521	243	122	64	45
	2.0		360	165	85	45	26
	2.5	518	274	123	61	30	22
3.5	1.0			374	197	103	63
	1.5		450	229	110	56	41
	2.0		308	143	73	38	24
	2.5	415	247	111	54	26	19
4.0	1.0			356	175	95	58
	1.5		391	178	96	38	34
	2.0		281	127	65	34	22
	2.5	395	218	96	47	23	17
4.5	1.0			320	167	88	53
	1.5		354	173	86	41	30
	2.0		265	114	60	30	20
	2.5	356	183	85	39	21	16
5.0	1.0			277	148	81	50
	1.5		315	155	67	37	27
	2.0	448	241	106	55	28	18
	2.5	303	167	75	36	19	13
5.5	1.0			252	142	72	37
	1.5		298	150	73	34	25
	2.0	408	112	103	50	25	16
	2.5	280	164	73	37	17	12
6.0	1.0			250	131	66	34
	1.5	544	284	137	68	33	22
	2.0	389	189	93	46	23	15
	2.5	273	147	66	33	17	11
7.0	1.0			236	118	48	38
	1.5	503	262	122	60	31	20
	2.0	419	189	78	41	20	14
	2.5	236	138	60	29	15	10

Table 2-3(b) The completion times at fast Maillard reaction

Reactant concentration			Completion times (d)					
Xylose	Glycine	Na_2HPO_4	0	5	10	15	20	25
1.0	1.0	0.2				11.0	4.3	2.0
		0.3				7.5	3.5	1.7
		0.4				6.5	3.0	1.4
	1.5	0.2		21.0	11.0	5.5	2.9	1.5
		0.3		20.0	10.0	5.0	2.4	1.3
		0.4	60.0	20.0	9.0	4.5	2.1	1.1
	2.0	0.2	61.5	21.0	10.0	5.0	2.4	1.3
		0.3	52.5	18.0	8.0	4.0	2.1	1.1
		0.4	42.5	16.0	8.0	3.5	1.8	1.0
1.5	1.0	0.2			14.0	5.5	3.1	1.5
		0.3	61.0	21.5	12.0	5.0	2.6	1.3
		0.4	49.0	24.0	9.0	4.0	2.3	1.2
	1.5	0.2	47.0	19.0	8.5	4.0	2.4	1.1
		0.3	40.5	14.0	7.0	3.0	2.2	1.0
		0.4	34.0	14.0	6.5	3.0	2.0	0.9
	2.0	0.2	34.0	14.0	7.0	3.5	2.1	1.0
		0.3	30.5	11.5	6.0	2.5	1.8	0.8
		0.4	29.0	11.5	5.0	2.5	1.5	0.8
2.0	1.0	0.2	54.0	21.5	9.5	5.0	2.7	1.3
		0.3	44.0	19.5	8.0	4.0	2.2	1.1
		0.4	41.0	14.0	7.0	3.0	2.0	1.0
	1.5	0.2	33.0	14.0	7.0	3.0	2.2	1.0
		0.3	30.5	13.0	5.0	2.5	1.7	0.8
		0.4	25.5	11.5	5.0	2.5	1.5	0.7
	2.0	0.2	30.5	13.0	6.0	3.0	1.7	0.8
		0.3	25.0	10.0	4.5	2.0	1.4	0.7
		0.4	21.0	8.0	4.0	2.0	1.3	0.6
2.5	1.0	0.2	47.0	16.5	8.5	3.5	2.3	1.1
		0.3	33.5	13.0	6.5	2.5	1.8	0.9
		0.4	33.0	11.5	6.0	2.5	1.6	0.8
	1.5	0.2	32.0	13.0	6.0	2.5	1.6	0.8
		0.3	25.5	11.5	4.5	2.0	1.4	0.7
		0.4	25.5	9.0	3.5	2.0	1.2	0.6
	2.0	0.2	27.0	10.0	5.0	2.0	1.4	0.7
		0.3	21.5	7.0	3.5	1.5	1.2	0.6
		0.4	18.5	6.5	3.5	1.5	1.0	0.5

The reaction rate of all conditions was shown temperature dependence according to the Arrhenius relationship. A relationship between reaction rate (r_{max}) and temperature (1/T) expressed by Arrhenius function showed high association of $R^2 > 0.9503$. Figure 8 shows representative Arrhenius plot for concentration conditions of Xy = 2.0 M, Gl = 2.0 M, and DH = 0.3 M. The reaction rate was increased as increasing of reaction temperature and concentration of reactant. Additionally, the range of E_a at each color channel (Red, Green, Blue) was 80–145 kJ/M, 79–117 kJ/M, 86–106 kJ/M, respectively. And the all parameter of the Arrhenius functions summarized in Table 2-4.

These E_a values are similar to the reported $E_a$values for each food quality losses. The typical E_a values for food quality losses were 41.84–104.60, 83.68–125.52, and 83.68–251.04 kJ/M for lipid oxidation, nutrient loss, and microbial growth, respectively (Labuza 1982). The Eα values for *Br.thermosphacta*, *Enterobacteriaceae*, and lactic acid bacteria in modified atmosphere packed beef were 85.69, 92.78, and 106.90 kJ/M, respectively (Vaikousi et al. 2009). And the typical E_a value for respiration rates occurring in fruits and vegetables was 29–79 kJ/M (Poças et al. 2008). Thus, these similar E_a values between the measured color change and the reported food quality losses show that the color change in Maillard reaction-based TTI can be applied to show the temperature history in the food quality change such as lipid oxidation, nutrient loss, and microbial growth.

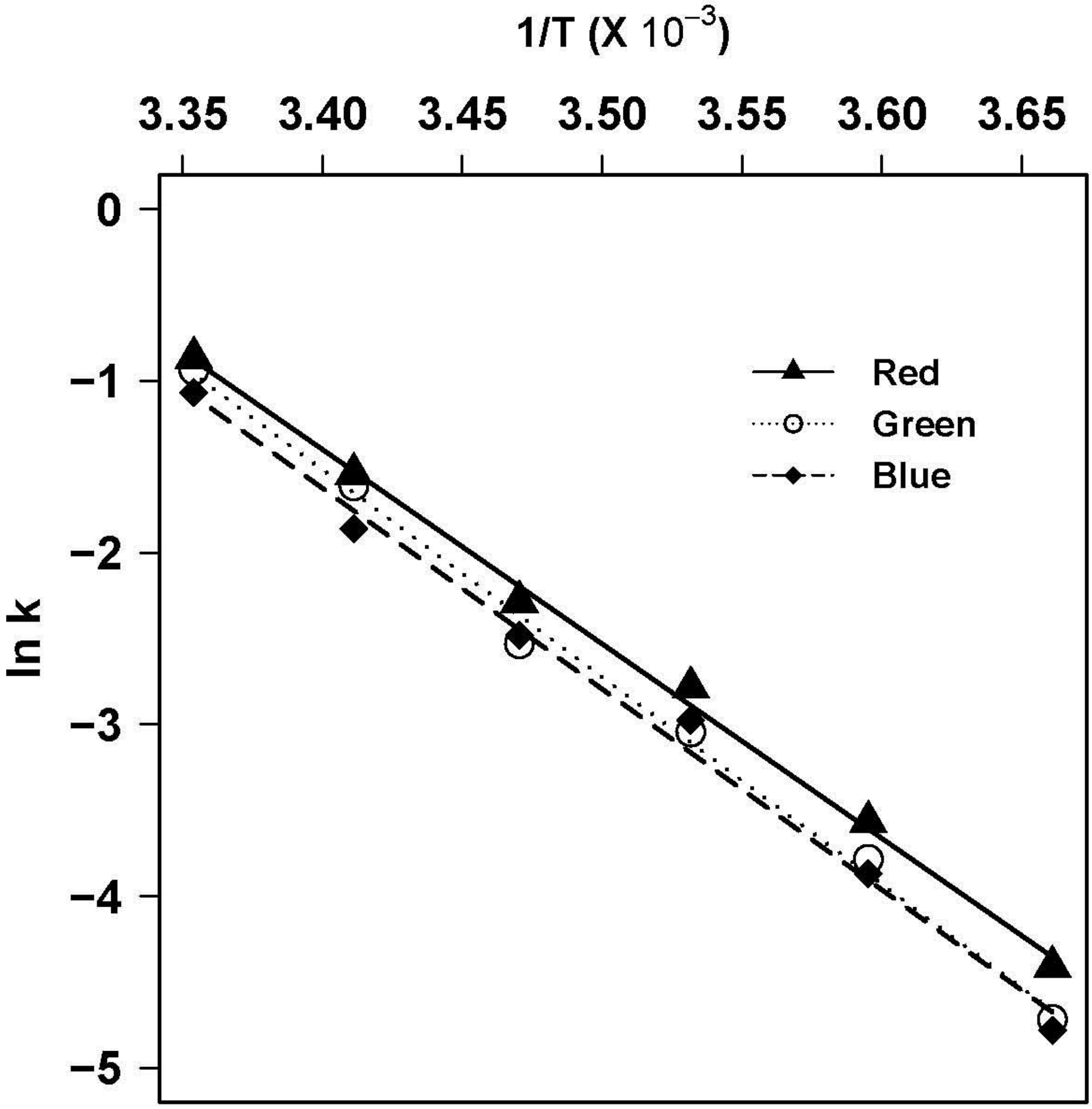


Fig. 2-8 Arrhenius plot at 2.0 M D-xylose, 2.0 M glycine, and 0.3 M Na_2HPO_4

Table 2-4 (a) The parameters of Arrhenius function (Ep. 2-3) at normal Maillard reaction

Reactant concentration		Red			Green			Blue		
Xylose	Glycine	Ea (kJ/M)	A (×10^{15})	R^2	Ea (kJ/M)	A (×10^{15})	R^2	Ea (kJ/M)	A (×10^{15})	R^2
1.0	1.0							94.23	2.2	0.9814
	1.5	145.63	1.66	0.9853	117.29	19562.85	0.996	107.96	983.22	0.9918
	2.0	106.74	394.66	0.9791	102.4	63.74	0.9948	99.67	54.83	0.9884
	2.5	99.87	21.28	0.9862	98.16	15.98	0.996	92.05	3.05	0.9896
1.5	1.0							106.26	386.79	0.9955
	1.5	101.37	32.22	0.9999	90.17	0.38	0.9903	105.83	500.41	0.99
	2.0	93.69	1.93	0.9881	98.1	16.06	0.9999	96.02	16.41	0.9833
	2.5	100.58	39.71	0.9797	91.45	1.32	0.9821	96.16	20.66	0.9791
2.0	1.0	110.6	1201.47	0.9821	79.12	0	0.9643	90.43	0.77	0.9985
	1.5	99.8	15.81	0.9874	86.3	0.1	0.9791	71.62	0	0.8518
	2.0	77.08	0	0.9738	84.83	0.09	0.9737	85.83	0.3	0.9466
	2.5	90.81	0.93	0.9718	94.35	5.97	0.983	94.58	12.51	0.967
2.5	1.0	103	54.7	0.9933	78.66	0	0.9906	99.71	39.89	0.993
	1.5	84.56	0.05	0.9853	92.24	1.55	0.9926	95.8	12.63	0.988
	2.0	95.83	7.01	0.9951	93.47	3.67	0.9896	86.34	0.4	0.9666
	2.5	98.02	23.9	0.9976	93.49	5.09	0.9876	90.07	2.45	0.9593
3.0	1.0	94.01	1.35	0.971	80.46	0.01	0.9774	95.72	8.02	0.9809
	1.5	88.44	0.26	0.9906	88.47	0.4	0.9943	94.39	8.49	0.9652
	2.0	88.64	0.43	0.9943	99.5	50.11	0.9849	81.06	0.05	0.9564
	2.5	96.08	11.76	0.9955	89.09	0.93	0.9846	87.42	0.9	0.975
3.5	1.0	94.11	1.74	0.9865	80.19	0.01	0.9829	66.02	0	0.8116
	1.5	90.17	0.55	0.9525	89.28	0.61	0.9914	88.46	0.74	0.9684
	2.0	95.62	8.59	0.9953	96.61	16.43	0.9496	81.81	0.07	0.9571
	2.5	92.06	2.31	0.9837	90.51	2.11	0.9855	76.97	0.01	0.9353
4.0	1.0	87.43	0.12	0.9861	86.29	0.11	0.9931	97.46	18.98	0.9789
	1.5	104.77	308.56	0.9787	97.1	18.23	0.9854	90.87	2.29	0.981
	2.0	92.14	2.4	0.9932	101.33	129.37	0.9845	88.19	1.05	0.9709
	2.5	94.08	6.69	0.9768	93.69	9.31	0.9774	77.31	0.02	0.9671
4.5	1.0	94.22	2.42	0.9957	88.46	0.3	0.9948	98.34	34	0.9947
	1.5	106.55	643.27	0.9926	96.85	16.96	0.9875	88.66	1.01	0.9666
	2.0	97.79	27.05	0.9935	99.12	67.46	0.9935	95.08	21.54	0.9921
	2.5	91.13	2.2	0.9737	89.59	1.75	0.9805	85.8	0.61	0.9855
5.0	1.0	82.89	0.03	0.9999	87.24	0.21	0.9996	93.41	5.25	0.9861
	1.5	97.66	20.07	0.9751	93.51	5.27	0.992	96.02	26.39	0.9503
	2.0	91.81	2.49	0.9924	102	257.02	0.9872	85.78	0.56	0.9837
	2.5	91.68	3.44	0.9837	93.25	9.55	0.996	93.3	15.31	0.994
5.5	1.0	89.33	0.35	0.9471	92.21	1.85	0.9771	92.37	3.34	0.9973
	1.5	92.55	2.42	0.9909	100.41	85.47	0.9925	86.86	0.61	0.9709
	2.0	90.95	2.1	0.9454	87.97	0.91	0.9649	78.92	0.04	0.9656
	2.5	95.73	17.42	0.9575	89.75	2.2	0.9838	98.02	126.9	0.9982
6.0	1.0	93.71	2.3	0.9903	102.39	117.41	0.9974	90.42	1.54	0.9871
	1.5	98.92	33.91	0.9894	92.53	3.53	0.9894	88.89	1.48	0.9734
	2.0	96.42	19.31	0.9945	90.76	2.73	0.9934	93.22	14.31	0.9897
	2.5	92.41	4.77	0.9823	96.52	38.23	0.9915	88.25	1.99	0.9852
7.0	1.0	98.67	18.08	0.943	95.05	6.44	0.9769	97.21	23.53	0.9782
	1.5	102.24	154.53	0.9943	100.78	128.71	0.9969	99.25	111	0.9965
	2.0	110.75	7225.69	0.9809	99.72	125.54	0.9982	99.19	163.37	0.9988
	2.5	98.74	75.34	0.9923	97.13	52.62	0.9747	99.77	284.54	0.9888

Table 2-4(b) The parameters of Arrhenius function (Ep. 2-3) at fast reaction

Reactant concentration			Red			Green			Blue		
Xylose	Glycine	Na_2HPO_4	Ea (kJ/M)	A (×10^{15})	R^2	Ea (kJ/M)	A (×10^{15})	R^2	Ea (kJ/M)	A (×10^{15})	R^2
1.0	1.0	0.2	81.71	0.02	0.9657	97.25	11.41	0.9885	95.78	6.62	0.9806
		0.3	88.98	0.49	0.9863	101.01	63.76	0.9872	99.43	33.01	0.9914
		0.4	96.75	13.12	0.9795	109.85	2654.58	0.9936	99.99	47.42	0.9976
	1.5	0.2	89.00	0.58	0.9920	98.79	31.47	0.9880	98.15	24.55	0.9879
		0.3	97.05	19.15	0.9958	108.33	2043.75	0.9963	97.86	25.13	0.9902
		0.4	103.45	306.92	0.9948	105.96	825.42	0.9964	101.06	103.02	0.9940
	2.0	0.2	93.63	4.26	0.9832	106.08	780.09	0.9921	99.96	58.49	0.9945
		0.3	99.03	50.68	0.9741	104.71	528.83	0.9958	98.35	34.53	0.9956
		0.4	104.38	554.92	0.9920	112.07	12261.43	0.9888	102.89	264.26	0.9981
1.5	1.0	0.2	81.35	0.02	0.9734	95.10	6.78	0.9948	90.87	1.12	0.9937
		0.3	83.92	0.09	0.9726	97.52	21.83	0.9948	93.93	4.99	0.9983
		0.4	88.10	0.54	0.9831	101.77	149.14	0.9982	99.54	53.72	0.9990
	1.5	0.2	86.07	0.24	0.9978	101.41	130.46	0.9935	95.05	9.14	0.9884
		0.3	92.33	4.02	0.9933	108.43	2709.98	0.9985	98.71	43.25	0.9971
		0.4	96.16	21.58	0.9948	110.40	7137.54	0.9954	103.62	381.24	0.9922
	2.0	0.2	86.97	0.39	0.9807	105.74	889.34	0.9998	99.23	57.32	0.9976
		0.3	91.71	3.86	0.9901	97.44	37.13	0.9773	92.79	5.06	0.9916
		0.4	99.02	90.55	0.9905	104.19	742.22	0.9967	97.67	41.92	0.9897
2.0	1.0	0.2	79.99	0.02	0.9598	94.16	5.46	0.9901	90.25	1.03	0.9977
		0.3	87.88	0.57	0.9995	103.23	264.45	0.9912	91.20	1.71	0.9979
		0.4	96.82	26.64	0.9924	104.48	561.68	0.9984	99.39	65.08	0.9949
	1.5	0.2	92.61	4.34	0.9941	103.64	386.28	0.9991	102.52	213.77	0.9963
		0.3	95.57	19.44	0.9921	106.83	1689.59	0.9936	104.33	563.43	0.9926
		0.4	98.67	76.22	0.9968	109.68	6365.84	0.9948	105.82	1081.45	0.9942
	2.0	0.2	97.76	44.51	0.9989	105.08	847.47	0.9955	104.95	725.65	0.9938
		0.3	94.13	12.90	0.9971	100.37	147.14	0.9955	97.19	35.91	0.9933
		0.4	91.69	4.99	0.9851	101.01	228.93	0.9964	93.72	9.21	0.9952
2.5	1.0	0.2	85.79	0.24	0.9844	97.82	30.79	0.9960	92.13	2.71	0.9962
		0.3	94.31	9.78	0.9911	102.25	225.65	0.9977	98.99	52.45	0.9924
		0.4	93.17	6.82	0.9929	102.85	292.49	0.9997	99.96	81.70	0.9958
	1.5	0.2	91.62	3.44	0.9876	103.93	502.82	0.9863	98.10	37.34	0.9753
		0.3	96.07	26.53	0.9894	108.92	4622.67	0.9963	105.69	1052.03	0.9914
		0.4	91.73	4.79	0.9697	104.44	803.37	0.9946	105.43	1056.07	0.9890
	2.0	0.2	97.03	36.59	0.9937	105.70	1296.62	0.9917	99.85	102.01	0.9926
		0.3	96.44	39.07	0.9974	99.16	113.71	0.9921	94.57	13.89	0.9914
		0.4	95.20	24.94	0.9908	96.88	42.90	0.9965	90.69	2.81	0.9905

2.3.4. Development of a model for predicting color variation

The fitted parameters (r_{max}, T_{inf}, and Δcolor change) of Eq. 1 were successfully described as functions of reactant concentrations and temperatures by Eq. 2-4, with the corresponding fitted parameters and fit quality indices (R^2) summarized in Table 2-5. Thus, developed prediction formula (Eq. 2-4) allowed us to comprehensively and accurately predict Maillard reaction-induced color changes, as exemplified by the accurate (R^2 = 0.99) prediction of reaction completion times (Fig. 2-7).

At the results in prediction of completion time, the difference of between observed time and predicted time were increased at the lower temperature condition. But the completion time of color variation at the lower temperature is longer and slower. For this reason, the give considering to increased time at low temperature might not have a significant effect on the practical prediction. Actually, the average differences between observed and predicted completion times at normal reaction equaled 40, 19, 8, 4, 5, and 4 d at 5, 10, 15, 20, 25 and 30 ℃, respectively. And, at fast reaction, the average differences show 42, 16, 9, 9, 5, and 3 h at 0, 5, 10, 15, 20, and 25 ℃, respectively.

Table 2-5 (a) The Results of regression analysis for normal reaction (Ep. 2-4)

Parameter		Coefficients						R^2
		a_0	a_1	a_2	a_3	a_4	a_5	
ΔColor value	Red	79.5222	-27.9568	0.0244	-0.0060	2.4484	-0.0060	0.1755
	Green	4.2406	-1.5442	-0.0191	-0.0037	0.1327	0.0110	0.1157
	Blue	27.0873	-9.5134	-0.0152	-0.0070	0.8276	0.0081	0.1683
r_{max}	Red	-2201.7538	734.7414	0.4183	1.2939	-61.2422	0.1541	0.9666
	Green	-2520.2952	847.8908	0.7744	1.3954	-71.2882	0.0497	0.9815
	Blue	-1374.5975	445.7797	0.5711	1.3620	-35.9799	0.0641	0.9692
T_{inf}	Red	3567.8837	-1217.5584	-0.8855	-1.4507	103.9525	0.0016	0.9888
	Green	2875.7518	-974.7261	-0.9508	-1.4879	82.6412	0.0513	0.9900
	Blue	1032.4524	-323.3430	-0.9267	-1.3319	25.0602	0.0974	0.9632
Completion time		3082.8463	-1046.3278	-0.7671	-1.3710	88.8526	-0.0394	0.9906

Table 2-5 (b) The Results of regression analysis for fast reaction (Ep. 2-5)

Parameter		Coefficients							R^2
		b_0	b_1	b_2	b_3	b_4	b_5	b_6	
ΔColor value	Red	-263.5575	91.0739	0.1894	0.1016	0.0708	-7.8743	-0.0698	0.7503
	Green	-106.2071	37.0490	0.0449	0.0294	0.0148	-3.2388	-0.0155	0.4900
	Blue	66.6571	-23.7712	-0.0160	0.0002	0.0048	2.1114	0.0239	0.3190
r_{max}	Red	-276.3945	57.2439	1.2787	0.6071	0.3435	-1.5823	-0.4556	0.9857
	Green	-677.5012	194.8310	1.0268	0.7398	0.3617	-13.3730	-0.3025	0.9915
	Blue	-1054.8547	330.3389	0.8177	0.6317	0.3419	-25.5333	-0.2591	0.9911
T_{inf}	Red	6807.4487	-2369.2676	-0.3854	-0.5600	-0.3005	206.2232	-0.3101	0.9933
	Green	7508.3625	-2615.2808	-0.5159	-0.6626	-0.3430	227.8113	-0.2122	0.9948
	Blue	7988.2403	-2783.6476	-0.4293	-0.6703	-0.4290	242.5704	-0.2838	0.9938
Completion time		4785.0001	-1648.7689	-0.7919	-0.7956	-0.4509	142.0808	-0.0595	0.9951

2.3.5. Development of an application for predicting color variation

Although the color change in TTI is visible to the naked eyes, confirmation of the color change in TTI using only eyes have some limits such as perception difference by personal gap and absence of prediction information about color change. For this reason, the using of application for widely deployed smart-phone was considered as a method for confirmation with ease and precision about color change in TTI. Therefore, application of prototype was produced, and examined about usability. An application was produced using XCODE (8.2) as integrated development environment and Swift3.3 as programing language.

(a) Capture section (b) Analysis section

Fig. 2-9 A developed prototype smart-phone application to analysis of TTI color

A developed application for smartphone recognizes a concentration condition and a appeared color in TTI, and then predicts about subsequent color change based on inputted temperature condition. In application, the OCR (Optical Character Recognition) was used for recognition of concentration condition in TTI, and the rate of progression in color change and the time remaining to reaction completion were calculated by developed prediction formula.

Therefore, a developed application was utilized at recognition and analysis of a made TTI with concentration conditions of Xy = 1.5 M, Gl = 1.5 M, and DH = 0.3 M for actual possibility verification. The executed application takes a picture of TTI (Fig 1-a), and shows the recognized information of TTI (The upper side of Fig 1-b) and the predicted values (The underside of Fig 1-b). Therefore, an application screen provide various information such as the concentration conditions and the currently color in TTI without prior information about TTI. Moreover, the users can see that if the TTI had been exposed to 10 ℃, the currently color of TTI was progressed 79% and the predicted remaining to reaction completion was 48 hours. Thus, a prototype application for smartphone using developed numerical model had sufficient possibility as a method for recognition and prediction to assist the naked eyes about the color changes in TTI.

2.4. Conclusion

Color variations observed in the Maillard reaction between D-xylose, glycine, and Na_2HPO_4 (reaction accelerator) were investigated at various temperatures and reactant concentrations for novel TTI development. The reaction completion time decreased with increasing temperature and reactant concentration, revealing that Maillard reaction-based TTIs with high reaction time flexibility can be realized by adjusting the latter parameter. Moreover, the reaction completion time and color variation could be accurately predicted by the developed numerical model. Additionally, a developed application was designed to recognition and change prediction of color in TTI had sufficient possibility as assistance method to naked eyes.

Chapter 3 Application of a developed TTI into ripening and storage for melon

3.1. Introduction

Muskmelon (*Cucumis melo L.*) originated in Africa and southwest Asia, is one of the most popular dessert fruit worldwide due to its unique fragrance and high sugar content (Oh et al. 2011). In addition, because melon is a ripening fruit, the quality easily decreases by proceeding of metabolism even under postharvest period. There have been many reports on the quality changes in various melon species to investigate the effect of production, storage, and distribution. Vito and Mikal (1995) reported on the effect of storage temperature for six inodorus melon cultivars (cv. Amarelo, Golden Casaba, Honeydew, Honey Loupe, Juan Canary, and Paceco). Yang et al. (2003) studied the storage quality potential of three main Hami melon cultivars (cv. New Queen, 8601, and Kalakusai). Youn et al. (2009) investigated the effects of temperature variations during storage on the quality characteristics of muskmelons (*Cucumis melo L.*).

Although many studies on quality change of melon during storage and ripening, determination of melon quality is still difficult without destruction. Because melon shows very little change even on thick rind, various nondestructive techniques for determination of the internal quality of melon have been reported such as acoustic technology, dynamic technology, electrical and magnetic technology, X-ray and computed tomography, and near infrared (NIR) spectroscopy, etc. (Sun et al. 2010).

Those methods, however, are also difficult to use in a real site, because those methods need special and expensive equipment. Novel technologies for determining and/or estimation of internal quality of melon without destruction have been demanded for long time. To overcome the problem, we have focused on a relationship between changes in melon quality and exposed integrated temperature during growing and distribution. Since Time-Temperature Integrator/Indicator (TTI) using Maillard reaction studied in this study has high flexibility on reaction time, this TTI would be able to use for indication of quality change during storage and ripening of melon. In this chapter, we aimed to investigate the relationship between quality change of melon during storage and ripening and color change in the developed TTI for clarifying the availability of the TTI at the storage and ripening process of melon.

3.2. Materials and methods

3.2.1. Materials

Melons (Red-113, harvested at Furano in Hokkaido) having similar sizes and weights harvested on early September of 2016 and 2017 was purchased from producing district. The melons were harvested at commercial maturity of 55-60 days after anthesis. The average weight and equatorial diameter of the harvested melons in 2016 (M2016) was 2183.5 ± 157.0 g and 50.1 ± 1.3 cm, respectively. The average weight and equatorial diameter of the melons harvested in 2017 (M2017) were 2445.8 ± 133.5 g and 52.2 ± 1.2 cm, respectively.

3.2.2. Pretreatment of melon

All melon was treated by the washing with hot water and used MA (modified atmosphere) packaging storage. First of all, the pollutant such as soil or dust was removed from the melon using tap water. Then, the melons were washed by the hot water (60 ℃) for 30 sec. After hot water treatment, to remove excessive water from the surface of the melon, the melons were wiped with the clean paper towel. The dried melons were stored in the modified atmosphere/modified humidity (MA/MH) packaging plastic bags (Xtend, 815-CN51, StePac Ltd., Israel). Xtend® films are characterized by high moisture vapor transmission rates (MVTR) in comparison to conventional polyethylene and polypropylene films (Porat et al. 2009). Figure 3-1 shows representative melons during storage and ripening.

Fig. 3-1 The appearance of the melons during MA storage

3.2.3. Temperature conditions

Temperature conditions were selected taking into considerations of real situation of storage and ripening. The melons harvested in 2016 were stored at 5℃ for 1, 2, 3, and 4 weeks, and then ripened at 25 ℃ for 2-8 days by shifting from the cold chamber of 5℃ at each week. The melons harvested in 2017 were stored at 4℃ for 2, 4, 6, 7, and 8 weeks, and ripened at 20℃ for 2-8 days. As control samples, the melons harvested in 2016 and in 2017 were stored at 25℃ and 20℃, respectively, for only ripening.

3.2.4. Weight loss

Weight loss of whole melons was determined by the comparison between the initial weight and the weight after ripening. The weight loss was calculated as percentage loss of initial weight (Eq. 3-1).

$$\text{Weight loss (\%)} = \frac{W_i - W_f}{Wi} \times 100 \qquad \text{(Eq.3-1)}$$

where W_i and W_f denote initial weight and weight after storage and ripening, respectively.

3.2.5. Color of the pulp

Color value of the stored melon pulp was determined by the colorimeter (Minolta CR-400 chroma meter, Konica Minolta Sensing, Inc., Japan) using the CIE scale L^* (Lightness), a^* (Red-Green), and b^* (Yellow-Blue). Melons were longitudinally cut into 2 halves. Seeds were removed, and remaining pulp was then cut into 8 equal segments. The color was determined with random four positions in pulp and the average of the measurement values was used. The color difference (ΔE) was defined by Ep. 3-2 as the visible difference of color (Thatyane et al. 2012; Zhou et al. 2016).

$$\Delta E = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2} \qquad \text{(Eq.3-2)}$$

where ΔL^*, Δa^*, and Δb^* is difference of measurement values and initial values.

3.2.6. Soluble solids contents (SSC)

Melons for measurement were longitudinally cut into 2 halves. Seeds were removed, and remaining pulp was then cut into 8 equal segments. And then, the 3 segments chose randomly. The soluble solids contents (SSC) of the melon was determined for squeezed juice from the three positions of top, middle, and bottom in each chosen segments by using a Pocket Refractometer (Pal-1, Atago Co., Tokyo, Japan; unit = % Brix). The top, middle and bottom represented near the connection point with the stem, equatorial position, and near the blossom end, respectively.

3.2.7. Firmness

The firmness of the melon pulp was determined for the three position of top, middle, and bottom as well as the determination of the SSC. The firmness of the pulp melon was expressed as the force in Newton (N) applied by a flathead probe of 10 mm in diameter with the crosshead speed set at 0.5 mm/s using a Rheo Meter (NRM-2002J, Rheotech Corp., Japan). The firmness was determined three positions for each part, and the average of the triplicate measurement values was used.

3.3.8. Temperature dependence of quality change for melon

The temperature dependence of quality change for melon was expressed by Arrhenius function (Eq. 2-3 in Chapter 2). A k value is slope (a) in the fitted results of each quality change by a linear equation (Eq. 3-3).

$$\text{Quality change} = \text{a} \times \text{Time} + \text{b} \qquad \text{Eq. 3-3}$$

3.2.9. Time-temperature indicator (TTI)

PET (poly ethylene terephthalate) film was used as a material for making TTI. PET film is a safe material which is normally used for various food packaging. Moreover, PET film has high strength, crystal clarity, moisture and solvent resistance, and the barrier against oxygen and water vapor, etc. (Thomas et al., 2011). For this reason, PET was considered suitable material for the TTI. Two different size (35 × 25 mm and 50 × 35 mm) of the PET film pouches were prepared by using poly sealing apparatus (PC-200, Fuji Impulse, Japan). In the small pouch, an acrylic panel (5 × 20 mm) was attached and sealed with D-xylose solution (0.75 mL). The small pouch was inserted into the large pouch, and then the large pouch was sealed with glycine solution (0.75 mL). The schematic representation of preparation of the TTI was shown in Fig. 3-2.

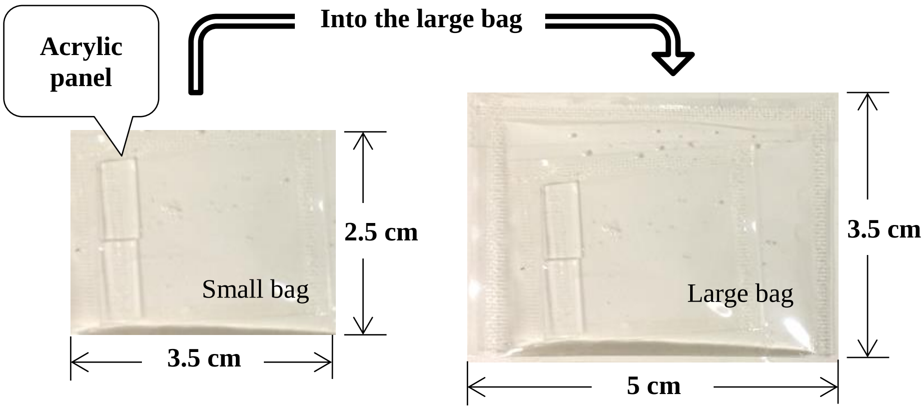


Fig. 3-2 Schematic representation of preparation of the TTI

3.2.10. Statistical analysis

SPSS (IBM SPSS Statistics 20, IBM, USA) was used to analyze the significance using one-way ANOVA. Significant differences among storage conditions were determined by Tukey's post hoc comparison test ($P < 0.05$).

3.3. Results and discussion

3.3.1. Changes in the appearance of the melon pulp during ripening period

Table 3-1(a) and 3-1(b) shows the changes in the appearance of the melon during ripening period for the trial in 2016 and the trial in 2017, respectively. The degradation of the appearance of the melon harvested in 2016 showed at the ripening conditions of 6 days at 25℃ after 2 weeks at 5℃, 2 days at 25℃ after 3 weeks at 5℃, and all melons after 4 weeks at 5℃. And the degradation of the appearance of the melon harvested in 2017 showed at the ripening conditions of 2 days at 20℃ after 7 weeks for 4℃ and all melons after 8 weeks for 4℃. Apparent degradation of the appearance exhibited on the 160℃·days and 220℃·days as integrated temperature for the melon harvested in 2016 and in 2017, respectively.

Table 3-1 (a) Changes in the appearance of the melon harvested in 2016 during ripening

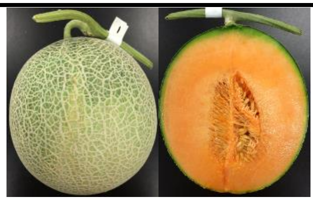
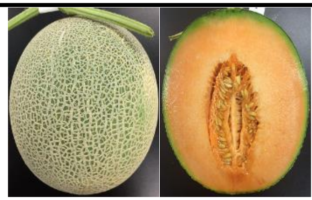
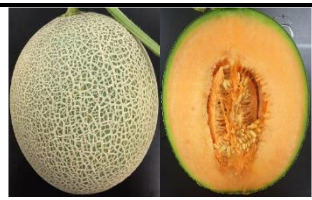
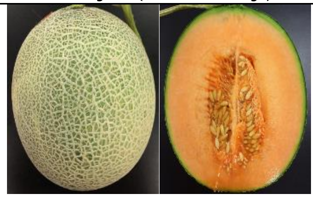
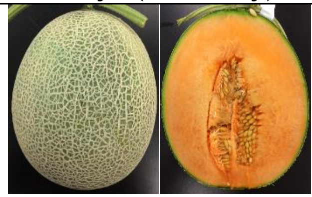
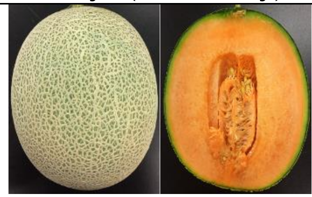
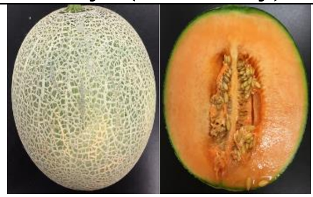
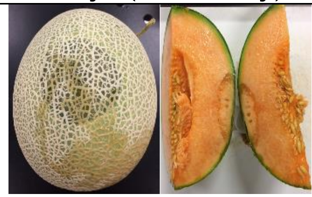
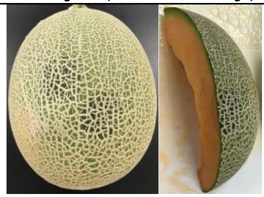
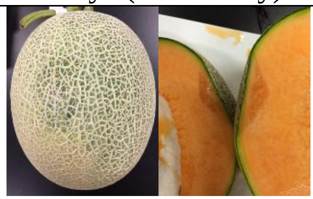
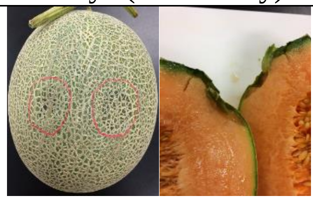
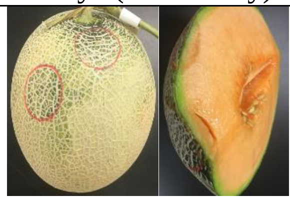
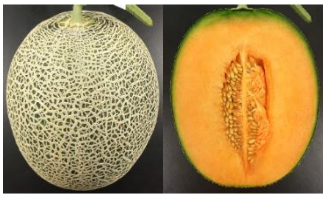
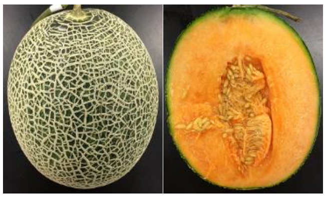
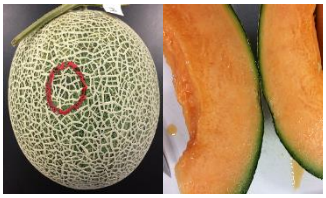
Only 25℃	 0 days	 2 days (48℃·day)	 4 days (95℃·day)	 6 days (138℃·day)	 8 days (182℃·day)
1 Weeks at 5℃	 0 days (44℃·day)	 3 days (112℃·day)	 6 days (179℃·day)	 8 days (222℃·day)	
2 Weeks at 5℃	 0 days (79℃·day)	 2 days (127℃·day)	 4 days (177℃·day)	 6 days (226℃·day)	
3 Weeks at 5℃	 0 days (116℃·day)	 2 days (164℃·day)	 5 days (241℃·day)		
4 Weeks at 5℃	 0 days (162℃·day)	 1 days (188℃·day)	 2 days (214℃·day)		

Table 3-1 (b) Changes in the appearance of the melon harvested in 2017 during ripening

Only 20℃	 0 days	 2 days (40℃·day)	 4 days (80℃·day)
	 6 days (120℃·day)	 8 days (160℃·day)	
2 Weeks at 4℃	 0 days (60℃·day)	 2 days (100℃·day)	 4 days (140℃·day)
4 Weeks at 4℃	 0 days (116℃·day)	 2 days (156℃·day)	 4 days (196℃·day)
6 Weeks at 4℃	 0 days (168℃·day)	 1 days (188℃·day)	 2 days (208℃·day)
7 Weeks at 4℃	 0 days (195℃·day)	 1 days (215℃·day)	 2 days (235℃·day)
8 Weeks at 4℃	 0 days (224℃·day)	 1 days (244℃·day)	

3.3.2. Weight loss

Weight loss by transpiration during ripening period was observed for all conditions. The weight loss rate was increased by the longer ripening period and the higher temperature. Fig. 3-3 shows weight loss of the melon harvested in M2016 (a) and harvested in M2017 (b). The increase of the weight loss at higher temperature was considered by increase in the transpiration under high temperature (Jamal and Chieri 2006). While the weight loss during ripening period at 20 and 25℃ was increased significantly, the weight loss after storage at 5℃ did not show significant difference among the storage period of 1-4 weeks. In contrast, the melon stored at 4℃ showed significant difference in the weight loss between 4 weeks and 6 weeks.

Moisture loss of crops is a major cause of postharvest deterioration and weight loss and lead to the economic loss if the commodity is sold by weight. Thus, sometimes weight loss is used as index of quality deterioration by nondestructive method. In wide range of horticultural crops, in general, weight loss of 3% to 10% may render unmarketable (Nunes and Emond 2007; Kasim and Kasim 2014). But weight loss does not correspond with quality deterioration. The melon harvested in 2017 showed degradation of the appearance despite weight loss in all conditions did not exceed either 3%. The degradation of the appearance of the melon harvested in 2016 was shown even at the around 1% of weight loss like in a ripened melon during 4 weeks at 5℃. Thus, TTI is able to useful for the decision of quality deterioration using the nondestructive method because the use of weight loss as index of quality deterioration is difficult.

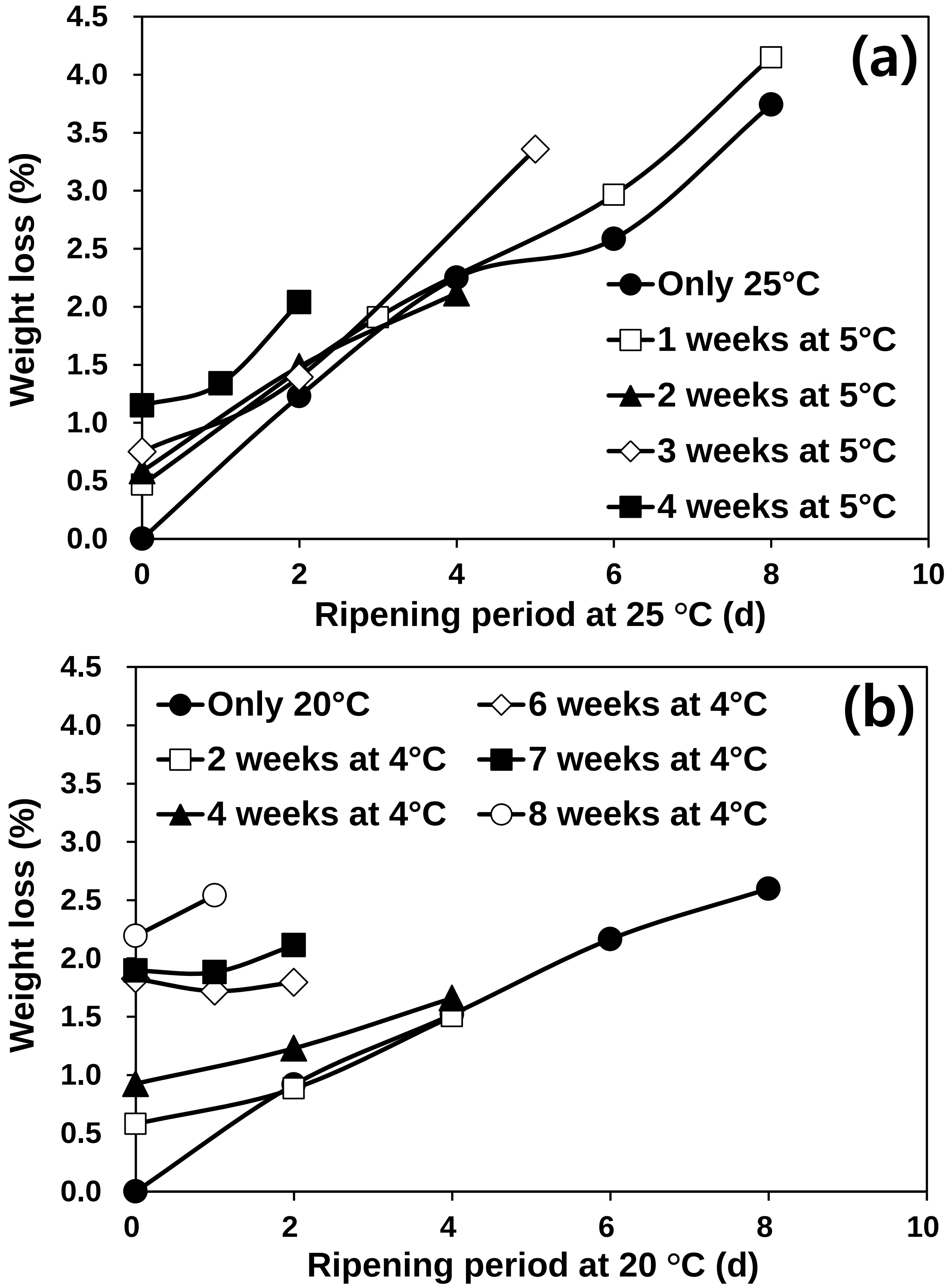


Fig. 3-3 The weight loss during ripen period at M2016 (a) and M2017 (b)

3.3.3. Color of the pulp

Fig. 3-4, 3-5, 3-6, and 3-7 shows changes in the color value of lightness (L^*), redness (a^*), yellowness (b^*), and color difference (ΔE), respectively. As shown in the Fig. 3-4, L^* values did not show significant variation ($p > 0.05$) by ripening period. In contrast, the a^* (Fig. 3-5) and b^* (Fig. 3-6) value significantly decreased during ripening period.

While the ΔE of the melons harvested in 2016 during ripening period at 25℃ was increased, the ΔE during storage period at 5℃ did not show the significant difference. The increase in the ΔE value at the higher temperature was attributed by the increase in the activity of polyphenol oxidase (PPO) and peroxidase (POD) (Chisari et al. 2008). Although the ΔE value increased with the longer period of storage and ripening, apparent difference was not recognized by naked eyes as shown in Table 3-1.

The changes in the a^*, b^* and ΔE value as a function of integrated temperatures are shown in Fig.3-8. The trend of the changes in the color values was described as a linear equation for a* and b* value, and a cubic equation for ΔE value. The a*, b* and ΔE values were decreased as the integrated temperature increased.

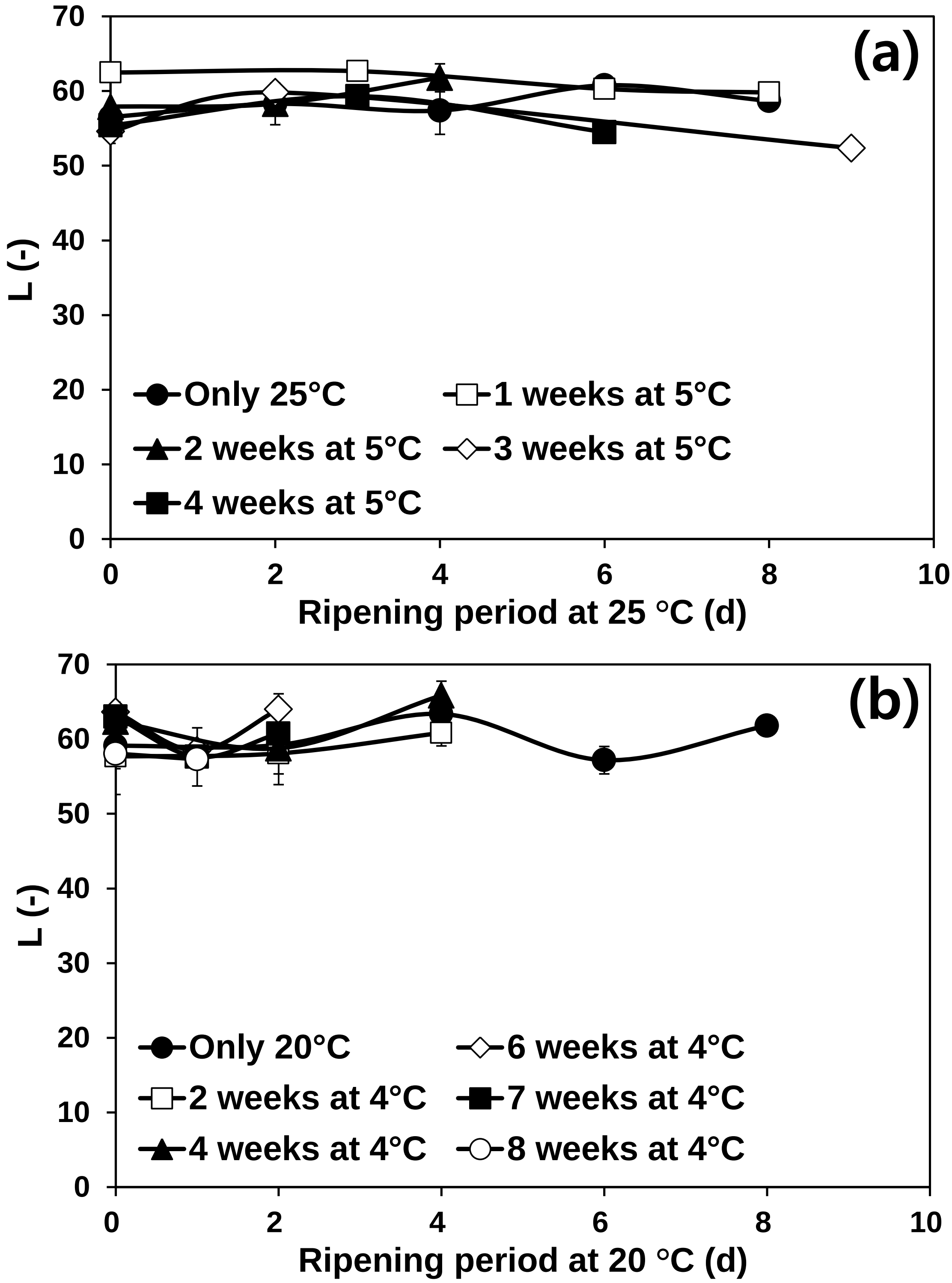


Fig. 3-4 The lightness (L) value variation by ripen period at M2016 (a) and M2017 (b)

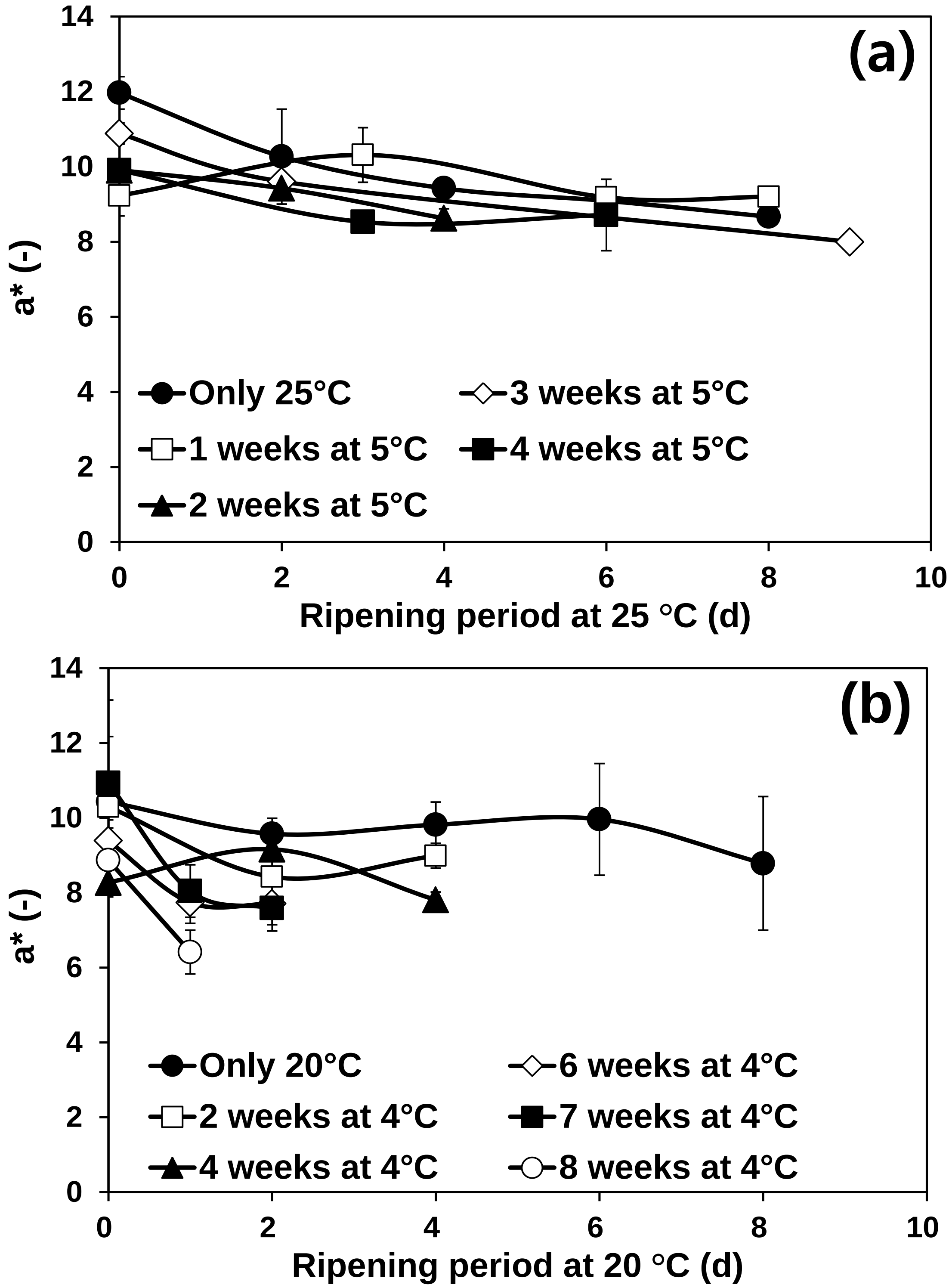


Fig. 3-5 The redness (a*) value variation by ripen period at M2016 (a) and M2017 (b)

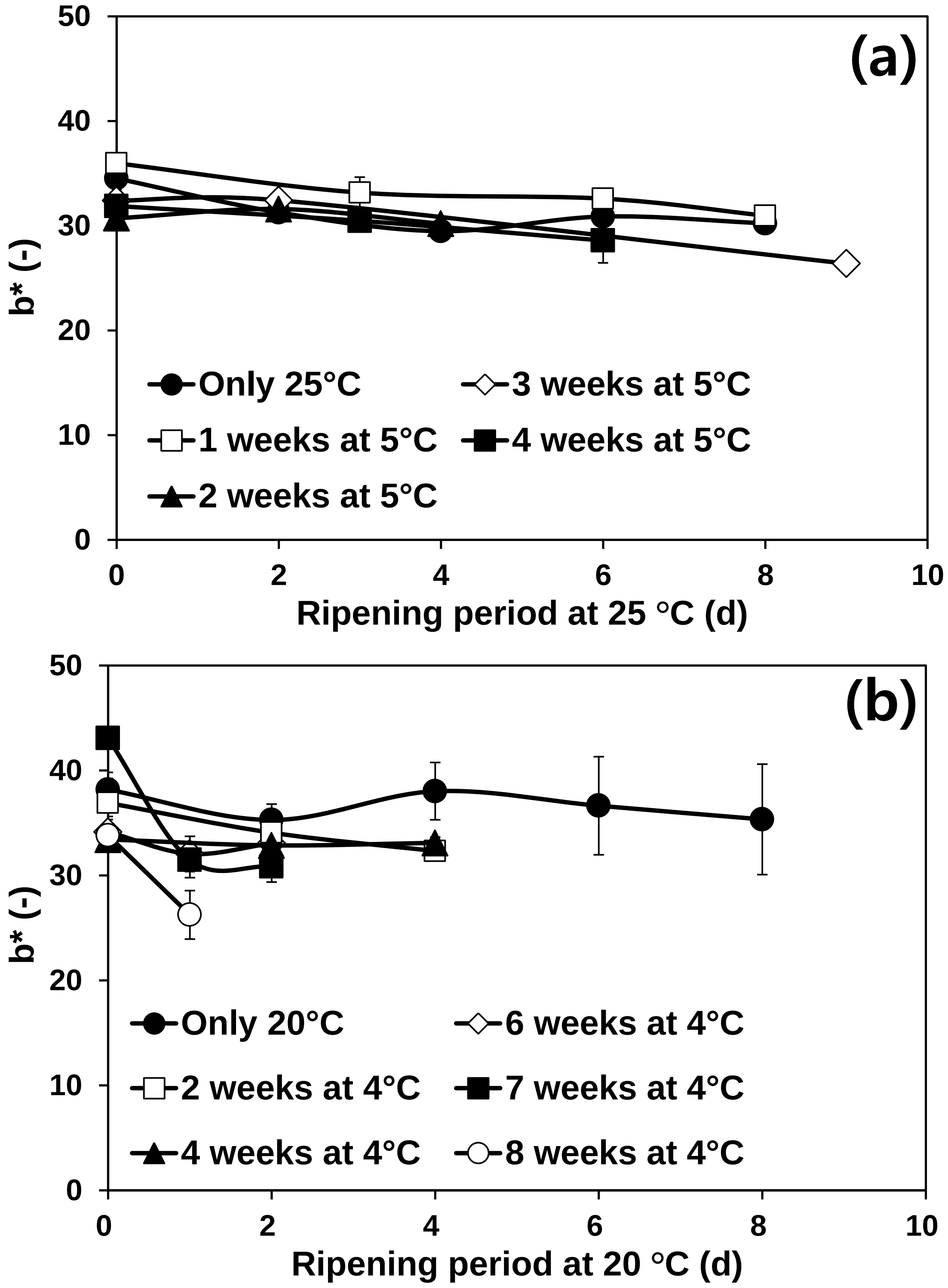


Fig. 3-6 The yellowness (b*) value variation by ripen period at M2016 (a) and M2017 (b)

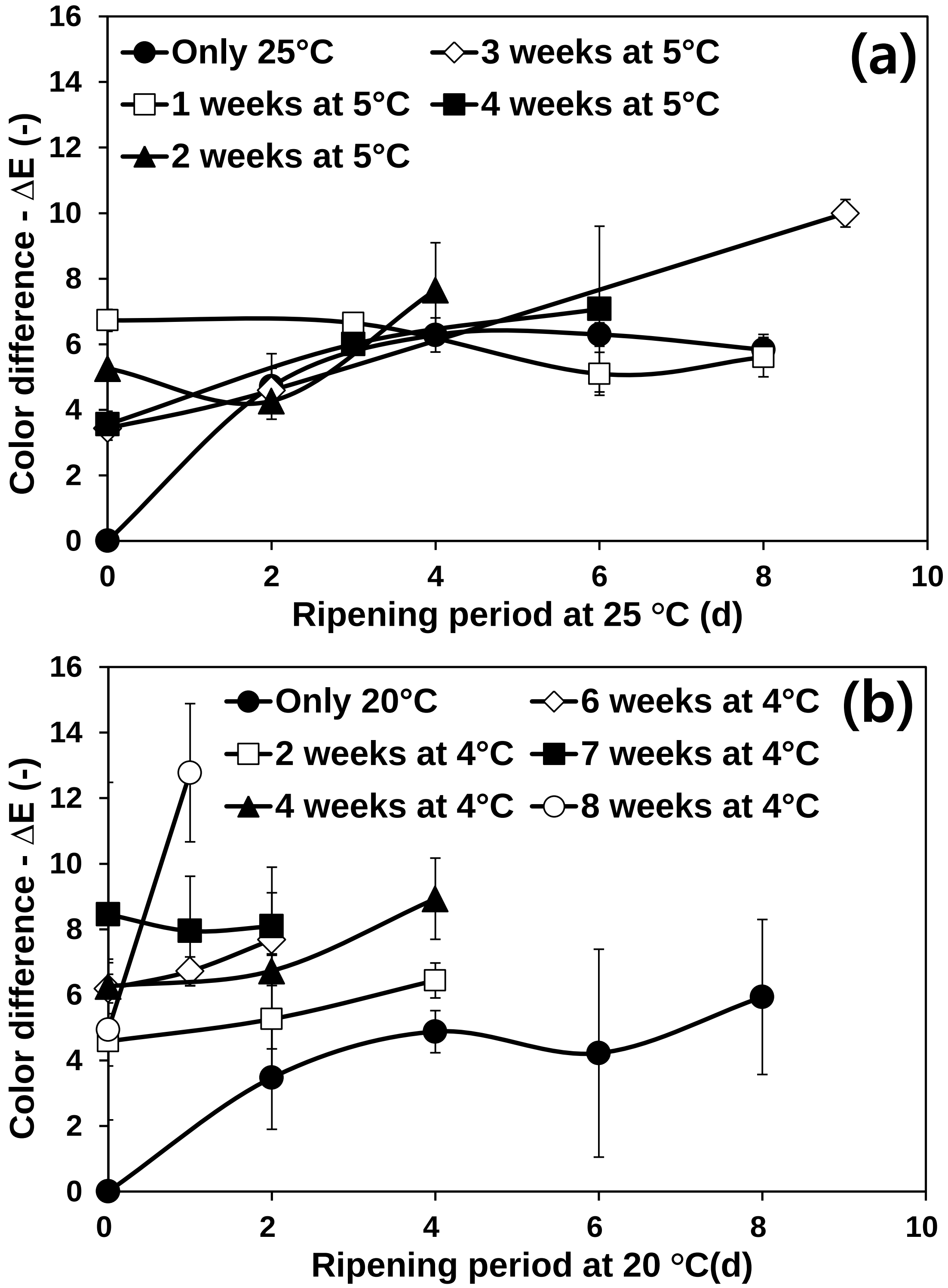


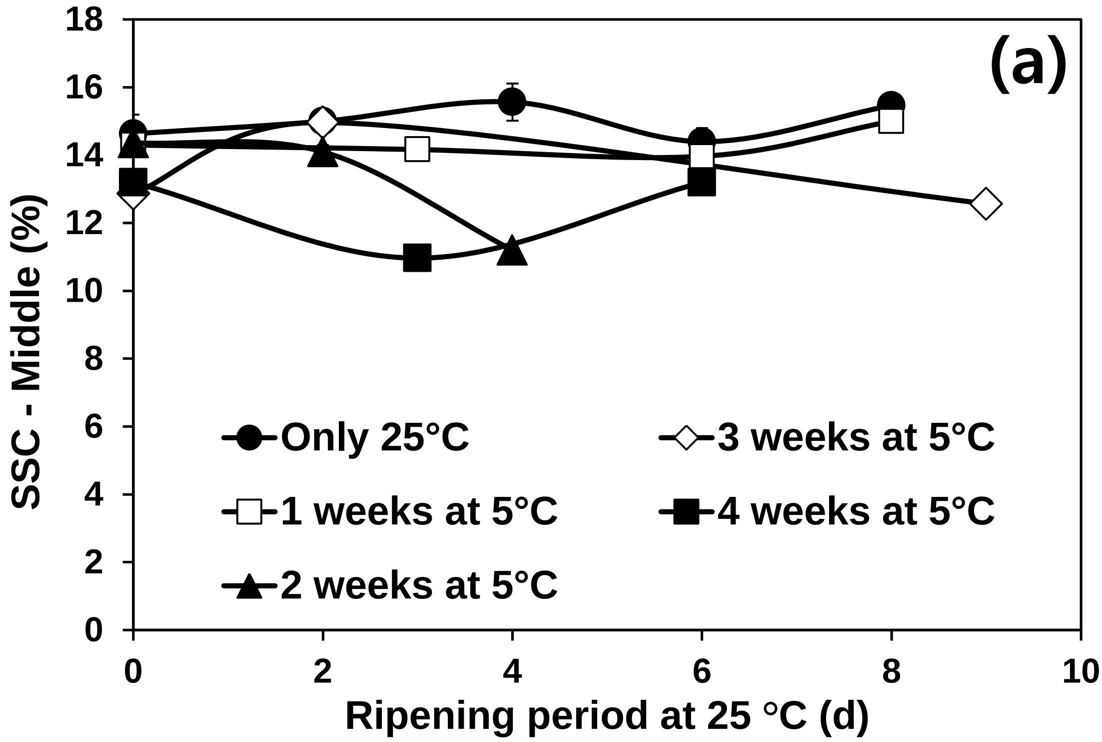
Fig. 3-7 The color difference (ΔE) value variation by ripen period at M2016 (a) and M2017 (b)

3.3.4. Soluble solids content (SSC)

The soluble solids content (SSC) decreased after storage and ripening. The SSC at the end of storage and ripening period was 9.4-14.0 %, while the initial value of SSC was 13.0-15.9 %. Fig. 3-8 shows changes in the SSC of different storage and ripening conditions. At M2016 by storage for 5℃ and ripening for 25℃, significant difference in SSC was not shown between the only ripened melon without storage and the ripened melon after storage during 1 week. These decreases of SSC during storage or ripening was attributed by the transformation of high molecular substances such as carbohydrate to sugar with tissue softening or the decomposition (Youn et al. 2009).

There was significant difference in SSC among the measurement position of the melon. The lowest value of SSC was shown at top position (Fig. 3-9), which was averagely 0.39-0.90 lower than those of other positions. This result was reported in Mizuno et al. (1971) and Nakayama (1968), also, that the lowest SSC was at top position (near the connection point with the stem), and the highest SSC was at middle position (equatorial position).

Fig. 3-10 shows the relationship between SSC that represents mean values of three different positions and integrated temperature. The linear relationship between the integrated temperature and SSC value was small as R^2 of 0.2869, despite the showing tendency of decrease at higher integrated temperature.



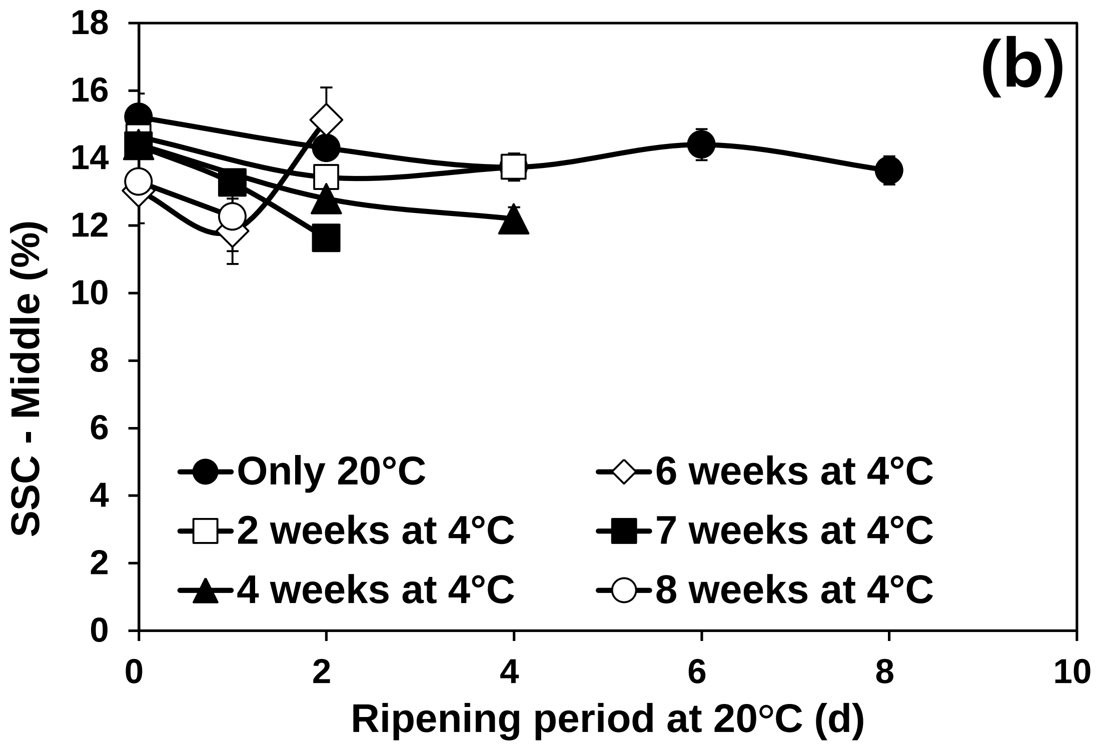


Fig. 3-8 The soluble solids contents (SSC) of middle position by ripen period at M2016 (a) and M2017 (b)

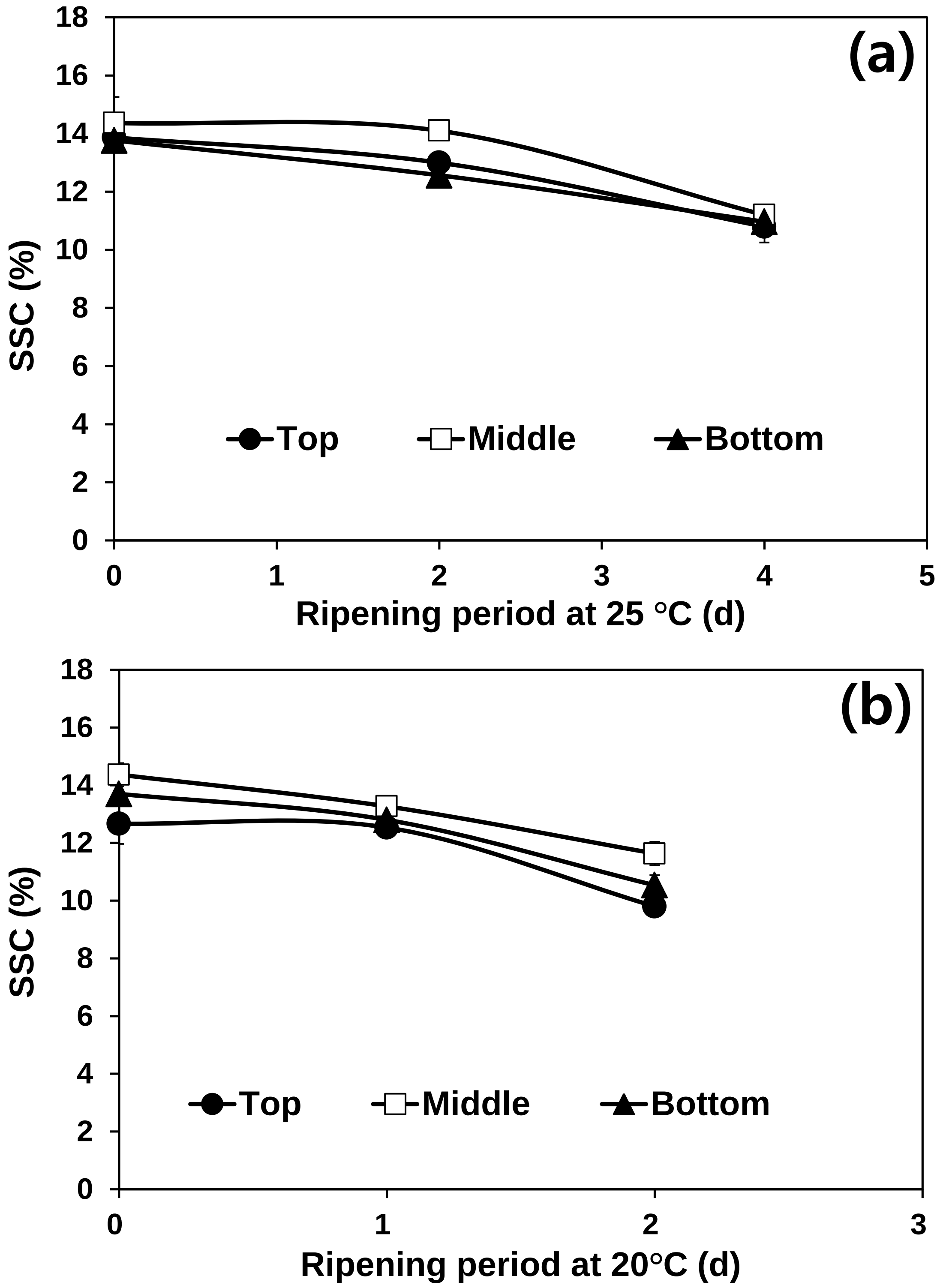


Fig. 3-9 The difference of soluble solids contents (SSC) by measurement position at M2016 (a) and M2017 (b)

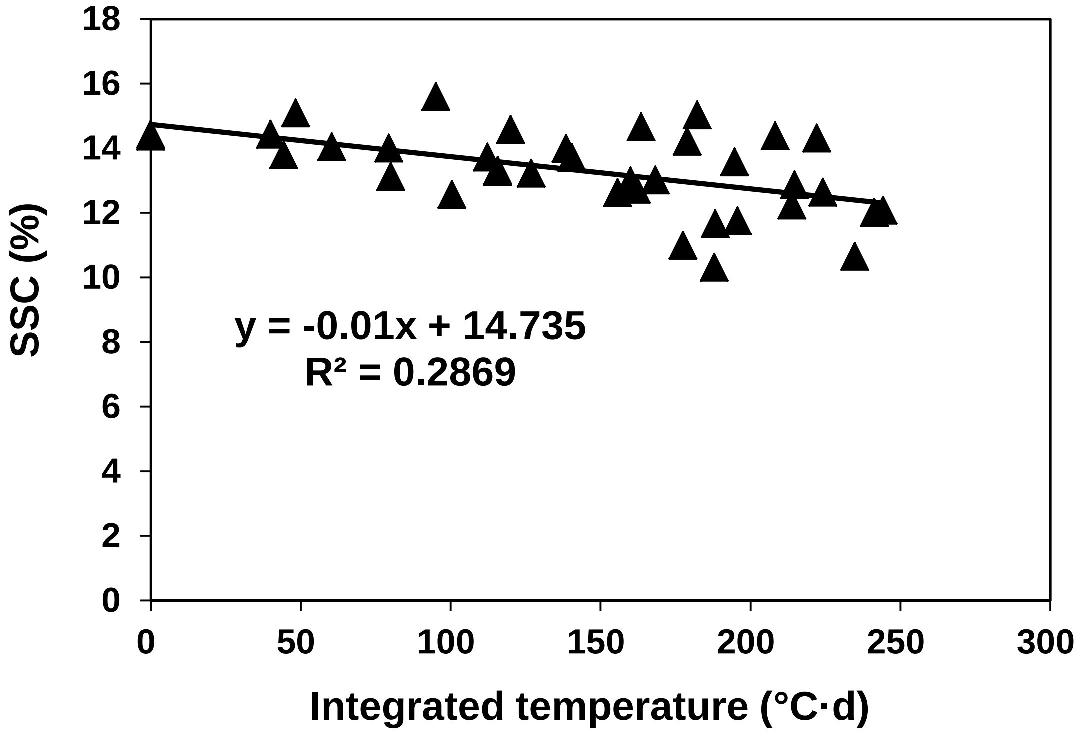


Fig 3-10 The relationship between SSC that represents mean values of three different positions and integrated temperature

3.3.5 Firmness

The firmness of fresh fruit is an important quality parameter for determination of the storage period after harvesting. The firmness of the melon gradually decreased during progress of ripening at all conditions. Average firmness of all measurement values was 4.24 N at initial, and decreased until 1.02-2.41 N after finish of storage and ripening. The changes in the firmness on the middle position in each condition shows in Fig. 3-11. The decrease of the firmness would be attributed by the transformation of pectin and hemi-cellulose(Oh et al. 2011).

There was significant difference in the firmness of the melon among the measurement position (Fig. 3-12). While the firmness of the melon was the highest in the top position, the firmness of the middle and bottom position showed similar values (Fig. 3-12). Paris et al. (2003) reported that firmness of melon differed among the measurement positions and was the highest at near stem end.

The changes in the firmness of the melon decreased as the integrated temperature increased (Fig. 3-13). In particular, when the integrated temperature increased by 100 ℃·d, the decrease rate of the firmness slowed and the significant difference among the firmness was disappeared. Therefore, when the integrated temperature reached around 100 ℃·d, ripening period would be finished.

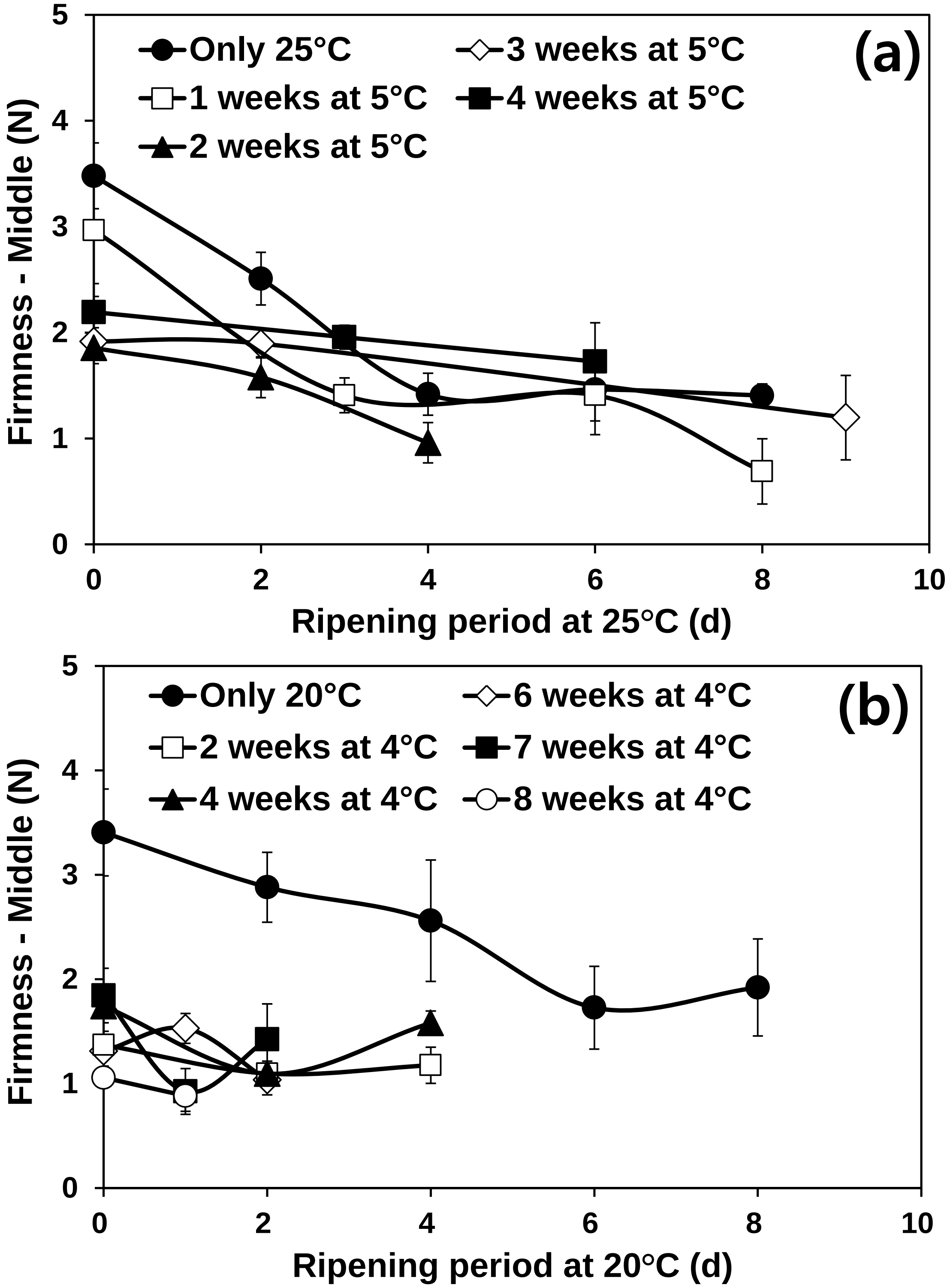


Fig. 3-11 The firmness variation of middle position by ripen period at M2016 (a) and M2017 (b)

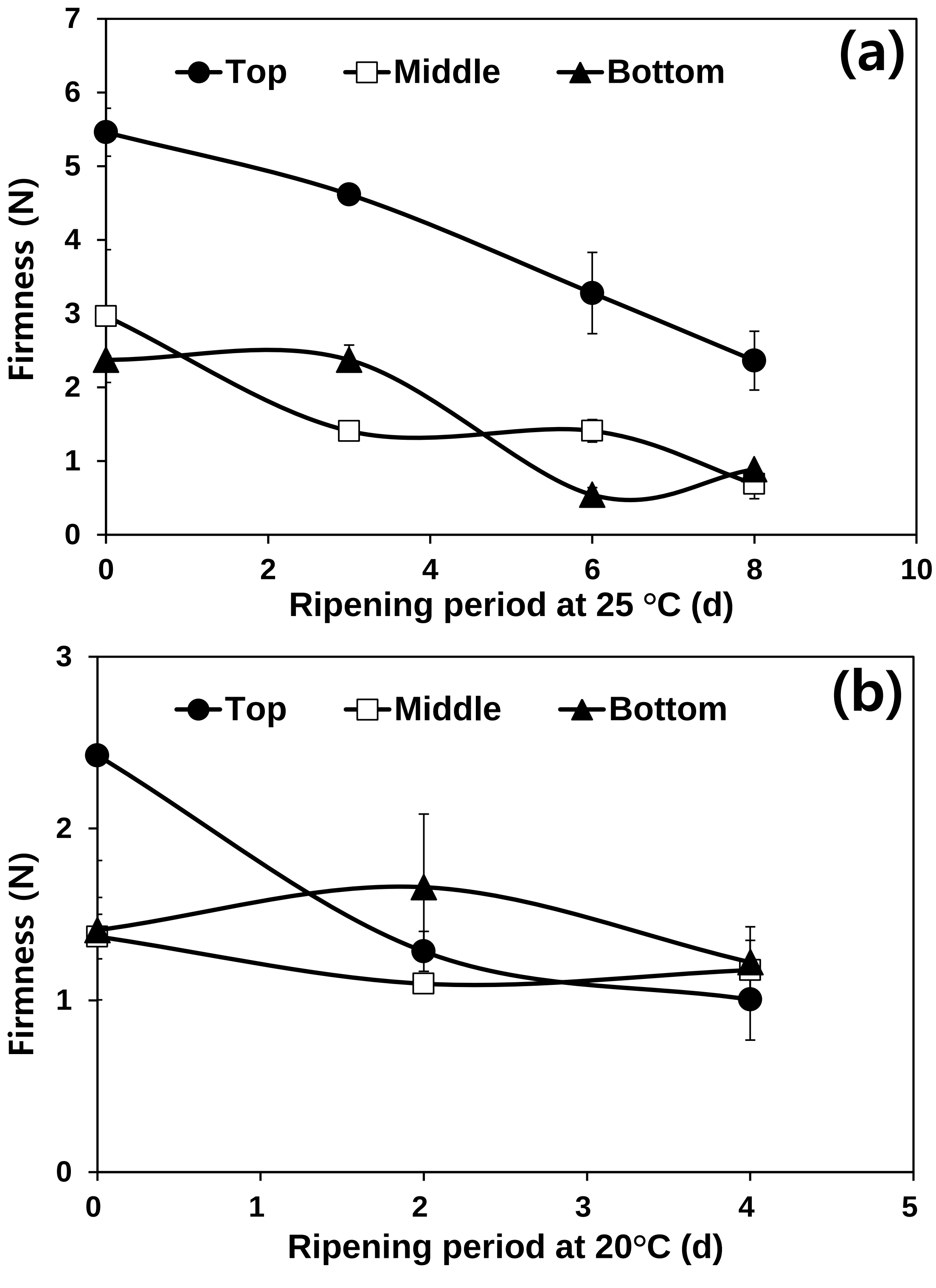


Fig. 3-12 The difference of firmness by measurement position at M2016 (a) and M2017 (b)

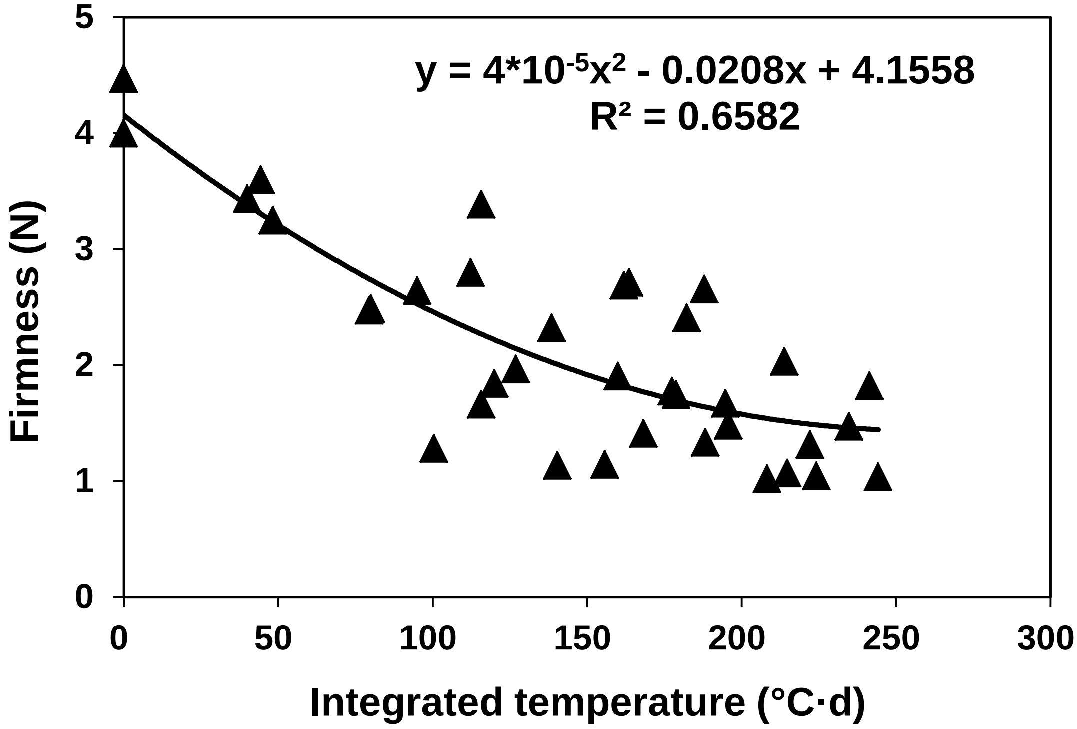


Fig. 3-13 The relationship between firmness that represents mean values of three different positions and integrated temperature

3.3.6. Temperature dependence of quality change for melon

The temperature dependence was examined about only weight loss and firmness, because significant difference by ripening temperature had at those qualities. Additionally, the slope of firmness quality change was calculated in the range from start day of storage to an initial measured day of firmness less than 2N, because significant difference by temperature condition among the firmness less than 2N was disappeared.

The quality change of weight loss and firmness was shown temperature dependence according to the Arrhenius relationship. A relationship between weight loss and ripening temperature (1/T) expressed by Arrhenius function showed high association of R^2 0.9887 (Fig. 3-14). And a relationship between firmness and ripening temperature expressed also high association of R^2 0.9463 (Fig. 3-15). Moreover, E_a at weight loss and firmness A were 85.23 kJ/M and 55.67 kJ/M, respectively. Those E_a values was similar with the range of E_a about color channel (Red, Green, Blue) of Chapter 2 such as 80–145 kJ/M, 79–117 kJ/M, 86–106 kJ/M, respectively. Thus, this similar E_a values between the quality change and the measured color change show that the color change in Maillard reaction-based TTI can be applied to show the quality change of weight loss and firmness.

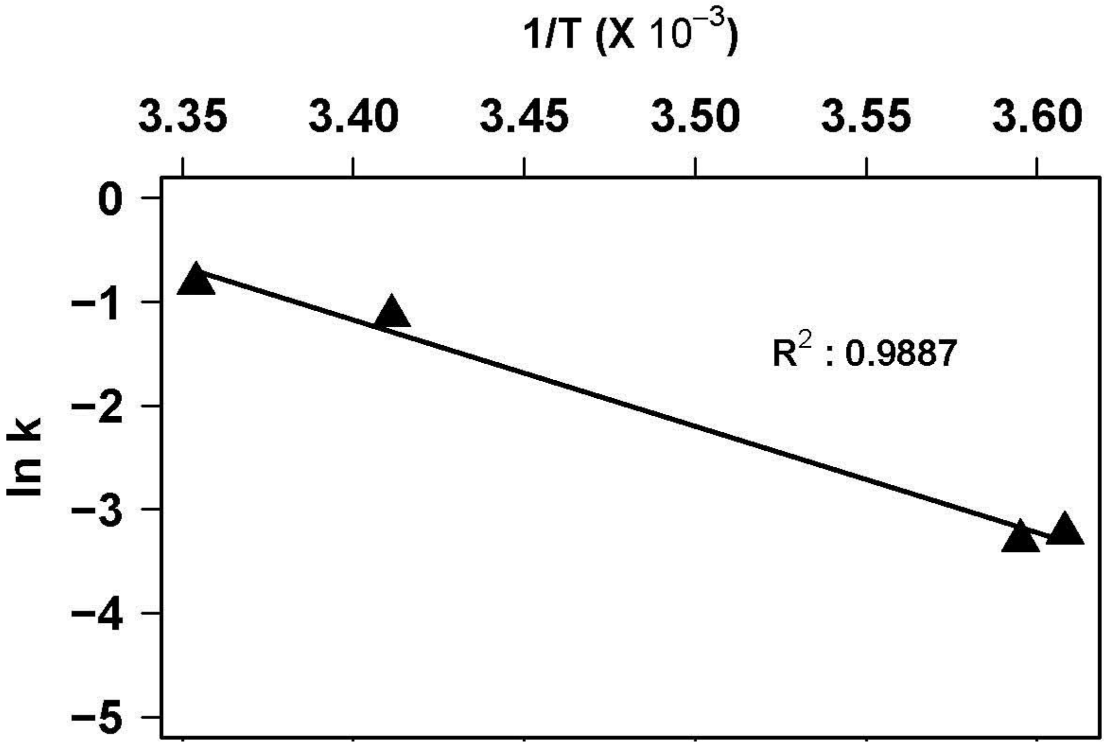


Fig. 3-14 Arrhenius plot between weight loss and ripening temperature

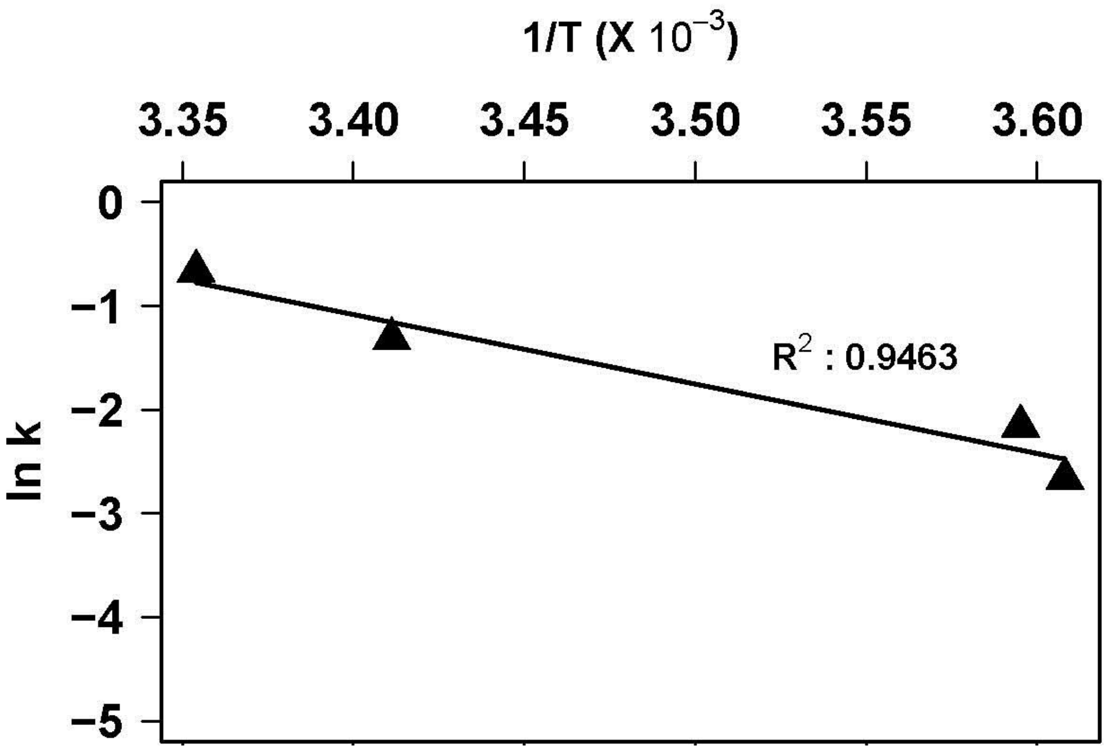


Fig. 3-15 Arrhenius plot between firmness and ripening temperature

3.3.7. Application of TTI (Time-Temperature Indicator)

The TTIs for estimation of the storage and ripening process of melon were developed as a decision support tool for determination of appropriate eating timing. The observed color variations of the TTIs during storage of melon were shown in Table 3-2. The TTI was developed on the basis of the quality change of the melons harvested in 2016, and applied for the melons harvested in 2017. The changes in the firmness were the most important parameter for determination of appropriate eating timing during storage period. Based on the changes in the firmness of melon during storage and ripening period, such as storage at 5 ℃ for 2-3 weeks and then ripening at 25 ℃ for 3-4 days (Fig. 3-12), the concentration of the Maillard-reaction based TTIs was determined as follows : (1) D-xylose 1.5 mol/L, glycine 1.0 mol/L, and Na_2HPO_4 0.2 mol/L, (2) D-xylose 1.0 mol/L, glycine 1.0 mol/L, and Na_2HPO_4 0.2 mol/L.

The developed TTIs clearly showed the color change. The color changes in the TTI appropriately reflected the changes in firmness of the melon during storage and ripening (Table 3-2). In addition, the brown color of TTI could reflect the weight loss of around 2 % and decrease the firmness by 2 N. The light blue and light brown before showing brown color allows to confirm and predict the quality change of the melon during storage and ripening. The colors of the TTIs enable users to choose fruits by personal preference. For example, those people who prefer softer texture could choose a melon with the TTI indicating brown color, and those people who prefer harder texture could choose a melon with the TTI indicating light blue or light brown color.

Table 3-2 The color variation in a made TTI (Time-Temperature indicator)

Only 20℃	 Initial TTI	 2 days	 4 days
	 6 days	 8 days	
After 2 weeks at 4℃	 0 days at 20℃	 2 days at 20℃	 4 days at 20℃
After 4 weeks at 4℃	 0 days at 20℃	 2 days at 20℃	 4 days at 20℃
After 6 weeks at 4℃	 0 days at 20℃	 1 days at 20℃	 2 days at 20℃
After 7 weeks at 4℃	 0 days at 20℃	 1 days at 20℃	 2 days at 20℃
After 8 weeks at 4℃	 0 days at 20℃	 1 days at 20℃	

3.4. Conclusion

The relationship between quality change of melon and color change in the made TTI was investigated for availability test of TTI at the storage and ripening process of melon. Therefore, the appeared brown color in TTI can express the weight loss of around 2 % and the firmness decrease to 2 N. Additionally, the quality change during storage and ripening of melon can be able to confirm and predict through the color change from light blue to light brown in TTI. Therefore, the actual made TTI (Time-Temperature Indicator) can be applied to show the quality change at the storage and ripening process of melon.

Chapter 4 Application of a developed TTI into shipping and storage for vacuum-packaged beef

4.1. Introduction

Consumption of beef in Japan is currently (in 2017) around 6 kg per person every year since 2010. Beef is domestically produced around 5 million ton and imported around 7 million ton every year. In particular, the beef imported from Australia dominates more than 50% in total import volume of beef. Most of the beef imported from Australia are shipped as chilling meats with vacuum-packaging for increase of shelf-life and shipping efficiency.

Seideman and Durland (1983) reported that vacuum-packaging was verified as a means of prolonging the shelf-life and palatability of beef. Lee and Yoon (2001) reported that growth of aerobic microorganism and discoloration in beef was inhibited by vacuum-packaging, and the shelf-life of the stored beef at 0℃ with vacuum-packaging was 66 days by sensory evaluation score. Hernández-Macedo et al. (2011) also reported that while vacuum-packaging increased shelf-life and shipping efficiency, might be induced quality degradation and/or spoilage by the anaerobic microorganism.

As described above, although vacuum-packaging improve quality and shelf-life of beef, the quality of the beef gradually degrades. Furthermore, vacuum-packaging is difficult to know spoilage state before packaging is opened. Thus, a special unique method is need for determination of the shelf-life or the progress of spoilage of beef. The spoilage of beef is appeared by various parameters such as pH, microorganism, color of flesh, odor, and storage condition, etc. (Hernández-Macedo et al. 2011). Although those parameters are determined for estimation of the shelf-life or the state of spoilage of beef, the shelf-life is mostly determined by odor and flesh color because the pH and the number of microorganism could not confirm by human sense. However, even the decisions by odor and/or flesh color have the personal difference, and can't predict remaining shelf-life or confirm temperature history.

For this reason, TTIs have been studied as a valuable tool for judging quality degradation and shelf-life of beef. Shin et al. (2006) studied a freshness indicator using change in pH on the flesh surface of beef and pork products during storage at 2℃. HarikliaVaikousi et al. (2009) reported that a developed microbial TTI can be used as an effective tool for monitoring microbial quality of fresh ground meat stored under modified atmosphere packaging (MAP) conditions. Ellouze and Augustin (2010) reported that a biological TTIs (eO)® could be applied as a quality management tool for a ground beef and a spiced cooked chicken slices packed under modified atmosphere. Kim et al. (2012) reported that a microbial TTI can be used in an intelligent packaging technology for representing some selected quality indices of ground beef. There were consistent relationships between changes in titratable acidity and corresponding quality indices, such as aerobic mesophilic bacterial counts, lactic acid bacterial counts, and freshness, regardless of storage temperature.

Although many types of TTIs have already been developed, the existing TTIs will be insufficient for long-term storage of beef under chilled temperature. There has not been developed any TTI that corresponds to changes in the quality of vacuum-packed beef during shipment at around 0℃. In this chapter, we aimed to investigate the applicability of the Maillard reaction based TTI for determination and/or estimation of the changes in quality of vacuum-packed chilled beef during shipping and storage. The color change in the Maillard reaction based TTI aimed to synchronize the changes in the shelf life of vacuum-packed chilled beef.

4.2. Materials and methods

4.2.1. Materials

The fresh vacuum packed beef samples that were striploin part were imported from Longford abattoir located in northern Tasmania, Australia. The vacuum packed beefs were shipped from Longford in Tasmania to Sapporo in Japan. The samples were stored at -0.5 ℃ during shipment with a temperature recorder (RS-14, ESPEC MIC Corp, Japan) and a trial Maillard reaction based TTI (Fig. 4-1). The shipping test was conducted twice and the samples after arriving at Sapporo were stored at -0.5 and 4 ℃ in our laboratory. Triplicate samples were used for each sampling time for determination of the beef quality. The initial properties of beef shows in Table 4-1.

Fig. 4-1 State of packing after arrival to Sapporo

Table 4-1 The initial properties of beef

	Shipping period	Storage temperature (℃)	Number	Average Weight (g)	Average surface area (cm^2)	pH	Microbiological (log CFU/cm^2)
Group A	16-12-16 ~ 17-01-24 (39 days)	-0.5	24	2438.03 ± 374.84	1326.53 ± 242.10	5.68	1.79
Group B	17-02-15 ~ 17-03-27 (40 days)	4	22	1927.74 ± 265.71	930.37 ± 128.24	5.64	1.54

4.2.2. Drip loss

The drip loss was determined by the ratio of the total weight of sample package and the difference between the total weight and meat weight. The drip loss was defined as follows:

$$\text{Drip loss} = \frac{W_0 - W_m}{W_0} \times 100 \qquad \text{(Eq.4-1)}$$

where W_0 is total weight and W_m is sample meat weight.

4.2.3. pH

The pH was determined on the three lean spots for each meat sample using pH meter (D-51, HORIBA, Japan) with ISFET (ion-sensitive field-effect transistor) pH electrode (0040-10D, HORIBA, Japan).

4.2.4. Color of flesh surface

Meat samples after opening the vacuum pack were exposed to ambient air condition for 10 min prior to analysis. The color value was determined on the lean surface by the colorimeter (Minolta CR-400 chroma meter, Konica Minolta Sensing, Inc., Japan) using the CIE scale L^* (Lightness), a^* (Red-Green), and b^* (Yellow-Blue). The color difference (ΔE) was defined as follows: (Rowe et al. 2004)

$$\Delta E = \sqrt{(L^* - L_0^*)^2 + (a^* - a_0^*)^2 + (b^* - b_0^*)^2} \quad \text{(Eq. 4-2)}$$

where L_0^*, a_0^*, and b_0^* as initial value that meant the first sample.

4.2.5. Microbiological analysis

Mesophilic viable count on the surface of the meat was determined by the whole surface of each beef sample. The sample taken out from vacuum pack was transferred to a sterile plastic bag and was massaged with 50 mL of 1% peptone water for 1 min. Appropriate serial dilutions (1:10) were performed with sterile 0.1% peptone water and were plated on duplicate tryptic soy agar (TSA) plates. The plates were then incubated at 20℃ for 5 days. All counts were expressed as log colony forming units per surface area of beef (log CFU/cm^2).

4.2.6. Shear force

The specimens (rectangular shape of 2×3×1 cm) were removed from each sample. Each specimen was sheared by three times perpendicular to the muscle fiber direction using a rheometer (NRM-2002J, FUDOH, Japan) with a straight-edged blade (0.35 mm thickness). The straight-edged blade compressed perpendicular to the muscle fiber direction and with a crosshead speed of 30 cm/min (Nihimura et al. 2008). The maximum peak force recorded was determined as the shear force.

4.2.7. Amount of 2-thiobarbituric acid reactive substance (TBARS)

Amount of 2-thiobarbituric acid reactive substance (TBARS) which is a byproduct of lipid peroxidation was determined by the procedure of Witte et al. (1970). A portion of 5 g sample meat was homogenized by the homogenizer (HG-92G, TAITEC, Japan) with pure water 15 mL. After filtration using Whatman filter paper No. 1, 1 mL of the filtrate was mixed with 2 mL of 20 mM 20% trichloroacetic (TCA) solution in 2-thiobarbituric acid (TBA), and was reacted at 100℃ in a water bath for 15 min for color development. The solution after the color reaction was filtered by 0.45 μm syringe filter (25AS045AN, ADVANTEC, Japan). The purified solution was dispensed into a 96 well microplate. The color was deteimned at 530 nm in a microplate absorbance reader (iMark, BIORAD, USA). The TBA value was defined as mg malonaldehyde (MDA) per kg of meat.

4.2.8. Cooking loss

The meat specimen (ca. 200 g) with the thickness for 15 mm was cooked in an oven at 230 ℃ until the internal temperature reached by 70 ℃. K-type thermocouple connected with a data-logger (MCR-4TC, T&D Corp., Japan) was placed in the center of specimen to monitor the internal temperature. The weight of each sample was recorded before and after cooking. Cooking loss was defined as the percentage of the difference in weight (Mcardle et al. 2010).

4.2.9. Sensory evaluation

The sensory evaluation was examined for drip volume, vacuum state, odor, and appearance (color) (Shang 2016). Each sensory attribute was scored by a 4-point descriptive scale. The sensory assessment form used in the present study was shown in Table 2. In particular, odor and appearance were evaluated in right after opening the package and 10 min after opening, because persistent odor and color bloom should be taken into consideration. Furthermore, the appearance was compared with the AUS-MEAT beef meat standard color (Aus-Meat 2005).

Table 4-2 Sensory assessment form

<table>
<tr><td rowspan="7">Date :

Age :

Name :</td><td rowspan="2">Drip</td><td rowspan="2">Vacuum</td><td colspan="2">Odor</td><td colspan="2">Appearance</td></tr>
<tr><td>Initial</td><td>After 10 min</td><td>Initial</td><td>After 10 min</td></tr>
<tr><td>4) None</td><td>4) Complete</td><td>4) Fresh,
no off odor</td><td>4) Fresh,
no off odor</td><td>4) Very fresh,
Deep red</td><td>4) Very fresh,
Deep red</td></tr>
<tr><td>3) Slight</td><td>3) Good
vacuum</td><td>3) Slight sour</td><td>3) Slight sour</td><td>3) Light red</td><td>3) Light red</td></tr>
<tr><td>2) Acceptable</td><td>2) Moderate
vacuum</td><td>2) Moderate
sour</td><td>2) Moderate
sour</td><td>2) Slight
discolouration</td><td>2) Slight
discolouration</td></tr>
<tr><td>1) Heavy</td><td>1) Poor
vacuum</td><td>1) Strong sour</td><td>1) Strong sour</td><td>1) Poor</td><td>1) Poor</td></tr>
<tr><td>0) Extreme</td><td>0) No vacuum</td><td>0) Off odor</td><td>0) Off odor</td><td>0) Severe
discolouration</td><td>0) Severe
discolouration</td></tr>
<tr><td>Sample 1</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Sample 2</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Sample 3</td><td></td><td></td><td></td><td></td><td></td><td></td></tr>
<tr><td>Comment</td><td colspan="6"></td></tr>
</table>

4.2.10. Time-temperature indicator (TTI)

The TTI examined in this study was designed on the basis of the methods for the Maillard-type TTI developed by Yamamoto and Isshiki (2012) (fig. 4-2). The plastic pouch is divided into two sections, one of which contains D-xylose solution and another one contains glycine solution with K_2HPO_4. Since the separation of two section was sealed weakly, pushing the both sides strongly to break the seal and mix the both solutions to start Maillard reaction.

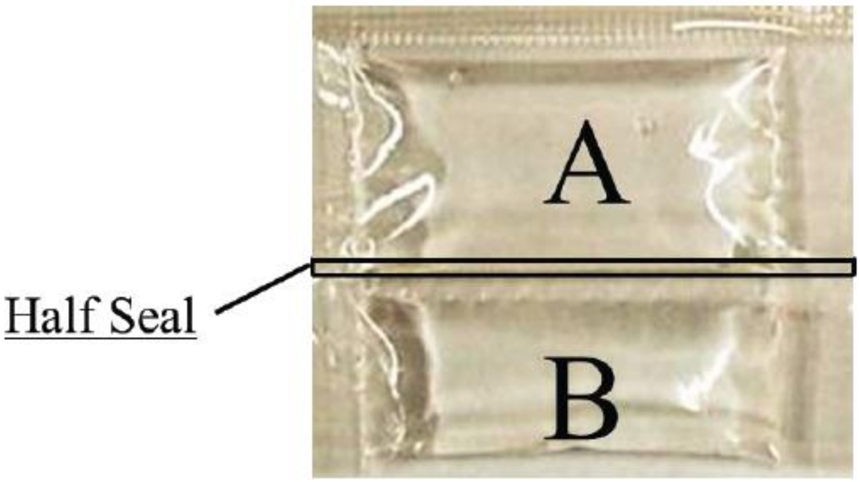


Fig. 4-2 A sample time temperature integrator/indicator (TTI) based on Maillard reaction proposed by Yamamoto and Isshiki (2012)

In addition, the concentration of TTIs for shipping was designed on the basis of storage period for 77 days at 0℃. The 77 day is the expiration date of the vacuum packaged chilled beef imported from Australia (All Japan Meat Industry Cooperative Associations 2015). The concentration of D-xylose, glycine, and Na_2HPO_4 for a TTI was followed Table 4-3.

Table 4-3 The concentration of reactant for TTIs

Reactant	Shipping		Storage at -0.5℃		Storage at 4℃	
	①	②	①	②	①	②
Xylose (mol/L)	1.0	5.0	1.0	3.0	7.0	1.0
Glycine (mol/L)	1.0	2.0	2.0	2.5	2.5	1.0
Na_2HPO_4 (mol/L)	0.2	0.02	0.1	0.01	0.0	0.2

4.2.11. Statistical analysis

SPSS (IBM SPSS Statistics 20, IBM, USA) was used to analyze the significance using one-way ANOVA. Significant differences among storage conditions were determined by Tukey's post hoc comparison test ($P < 0.05$).

4.3. Results and discussion

4.3.1. Changes in quality of beef after shipping

Aerobic plate count (APC) and pH of the beef samples significantly increased during shipping. Table 4-4 shows the results of determination of beef quality after shipping of group A and group B. Although the pH value of the beef surface increased from 5.68 to 5.94 after shipping, there was no significant difference (p-value > 0.05) before and after shipping.. The APC value also increased after shipping even under temperature of -0.5 ℃. Since the APC of beefs distributed in Japan in 2011 was reported 5.61-6.22 log CFU/cm^2 in average (Morita and Kobayashi 2016), which was higher than those of the measurement value. Moreover, the Microbiological self-management standard of Japanese Consumers Co-operative Union (JCCU, CO-OP) (2016) defines the criteria as less than APC of 7.00 log CFU/cm^2. Therefore, the beef of both the group A and B after shipping would arrive without microbiological degradation.

It was considered that group A and group B can be used as the comparison sample for measurement of quality difference by temperature during storage because the significant difference does not show between the group A and the group B (p-value > 0.05) at the quality after shipping.

TTIs exhibited arrival without microbiological degradation of beef the change to light yellow during shipping (Fig. 4-3). This change in light color expresses that the temperatures during shipping were constant with sufficiently low (Fig 4-4). In case of the changed of TTI color after shipping to brown or dark brown is mean that beef was exposed high-temperature and shipped with microbiological degradation.

Table 4-4 The quality value after shipping

	Shipping period	Drip loss (%)	pH	Microbiological (log CFU/cm^2)	Shear force (N)	TBARS (mg MDA/kg)	Cooking Loss (%)
Group A	16-12-16 ~ 17-01-24 (39 days)	3.98 ± 0.35	5.87 ± 0.12	4.88 ± 0.49	14.33 ± 1.39	1.87 ± 0.23	17.86 ± 1.55
Group B	17-02-15 ~ 17-03-27 (40 days)	3.95 ± 0.29	6.01 ± 0.09	5.09 ± 0.26	14.02 ± 0.97	1.74 ± 0.25	16.82 ± 1.82
p-value		0.92	0.14	0.60	0.91	0.29	0.57

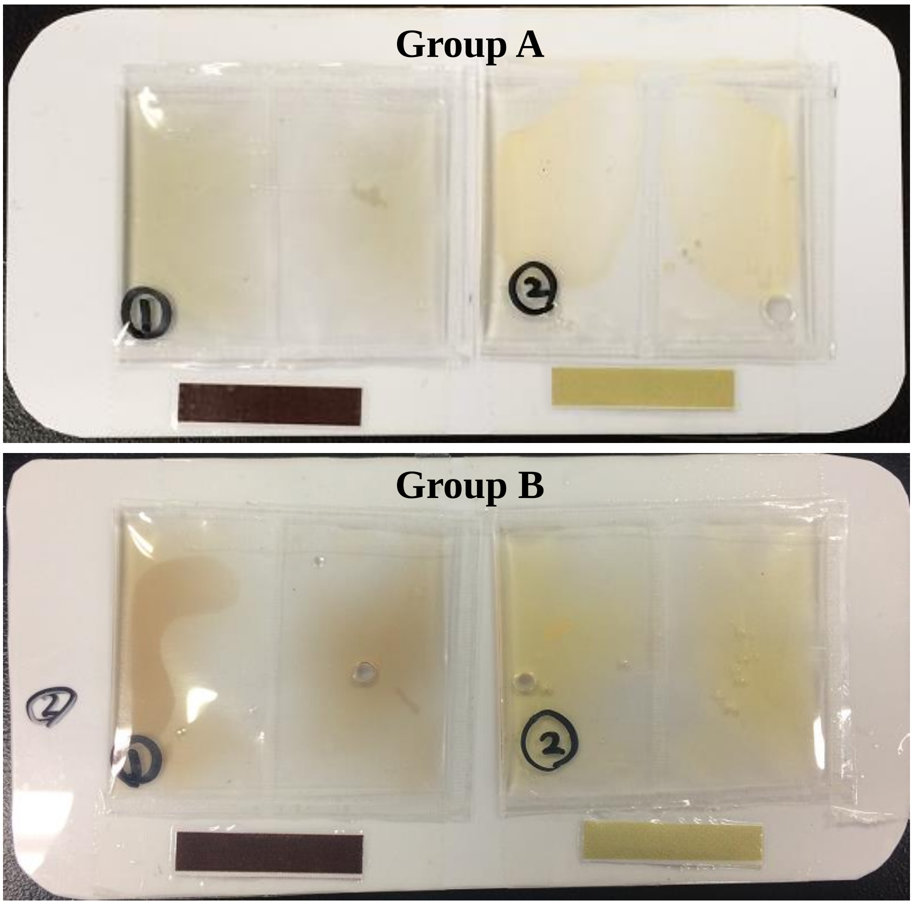


Fig. 4-3 The TTIs after shipping

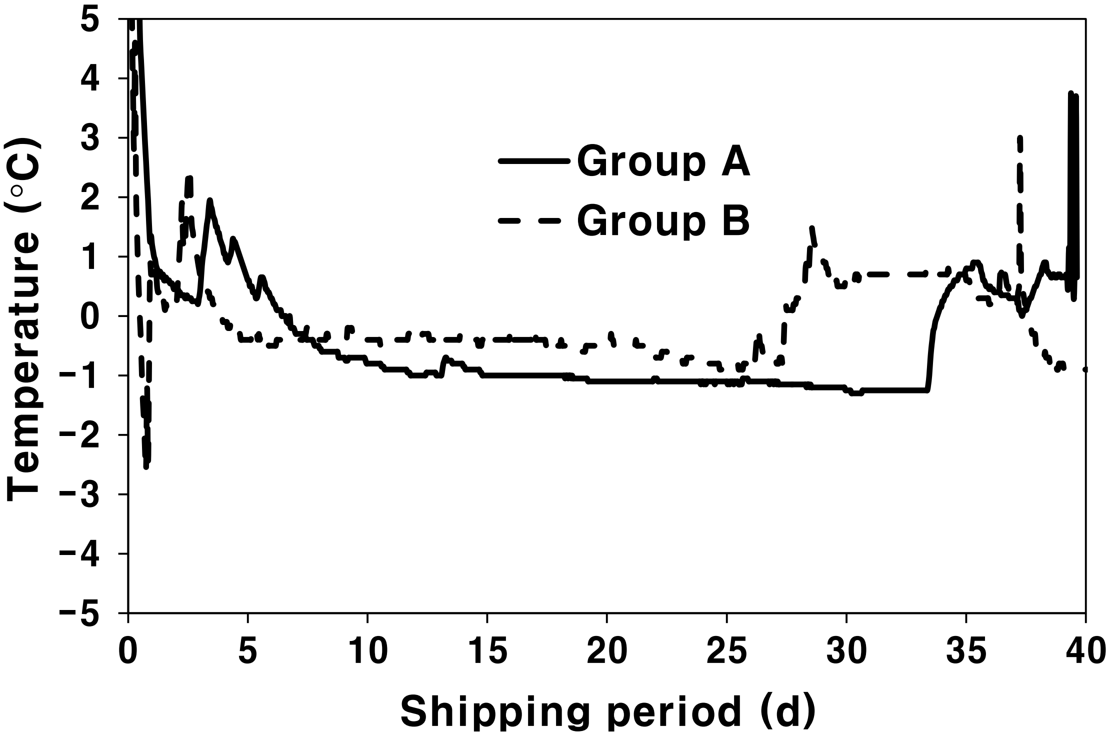


Fig. 4-4 The temperature variation during shipping period

4.3.2. Drip loss

The drip loss of vacuum-packaging beef increased as the storage period and the temperature increased (Fig. 4-5). Additionally, the changes in drip loss during storage at -0.5 and 4℃ showed the maximum value of 8.07% at day 86 and 6.69% at day 21, respectively.

The increase in drip loss as the storage period prolonged was also reported by various studies such as Lagerstedt et al. (2011), Laroque et al. (2008), and Payne et al. (1998). The increase in drip loss would be due to the decrease in the water-holding capacity of the muscle fiber structure and/or the denaturation of the proteins (Leygonie, Britz, and Hoffman 2012). In addition, the increase in drip loss at a higher temperature would be due to progress in proteolysis at higher storage temperature (Hertog-Meischke et al. 1998).

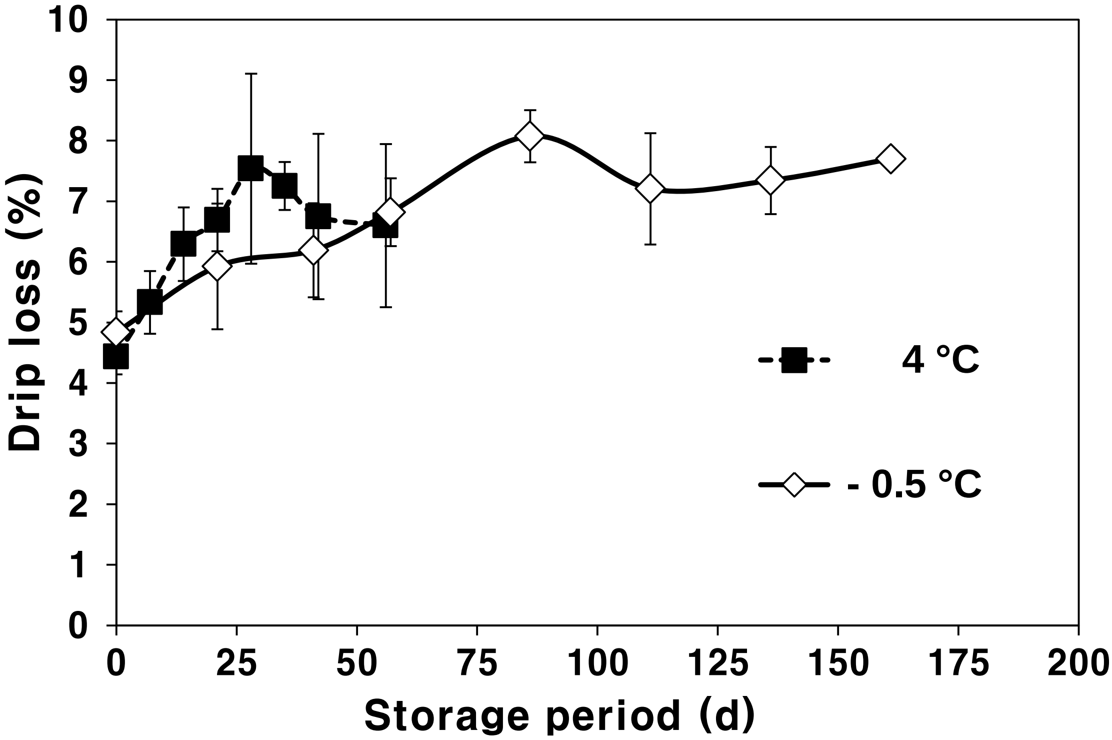


Fig. 4-5 Drip loss variation during storage period. Error bars indicate standard error (n=3)

4.3.3. pH, Color, TBARS

The pH, color values (L*, a*, and b*) and TBARS value showed no significant changes in all storage conditions (p-value > 0.05).

Ko and Yang (2001) reported that the increase of pH values during the progress of storage period is occurred because of accumulation of proteolytic products by enzymes and microorganisms in muscle. But the vacuum-packaging inhibits proliferation of the aerobic bacteria and the spoilage bacteria by lack oxygen (Beebe et al. 1976; Ercolini et al. 2011). Thus, the insignificant variation of the pH values was considered by the production inhibition of the proteolytic products and the aerobic bacteria under a vacuum-packaging.

The changes in color of meat during storage would be occurred by various parameters such as pH, temperature, oxygen tension, lipid oxidation, and stress before slaughter etc. (Bala et al. 1977; Jeong et al. 2006). The insignificant variation of color value was considered by inhibition those parameters under storage using vacuum-packaging and low-temperature around 0℃. In fact, Lee and Yoon (2001) and Moon (2005) reported about the insignificant variation of color value for the beef stored in 0℃ with vacuum-packaging. In addition, Samouris et al. (2002), Cayuela et al. (2004), and Popova et al. (2009) reported as no statistically significant differences in TBARS values of the vacuum-packaged meats between the first and last days of storage. This result is because that the low levels of oxygen by vacuum-packaging inhibit lipid oxidation (Smiddy et al. 2002; Cayuela et al. 2004)

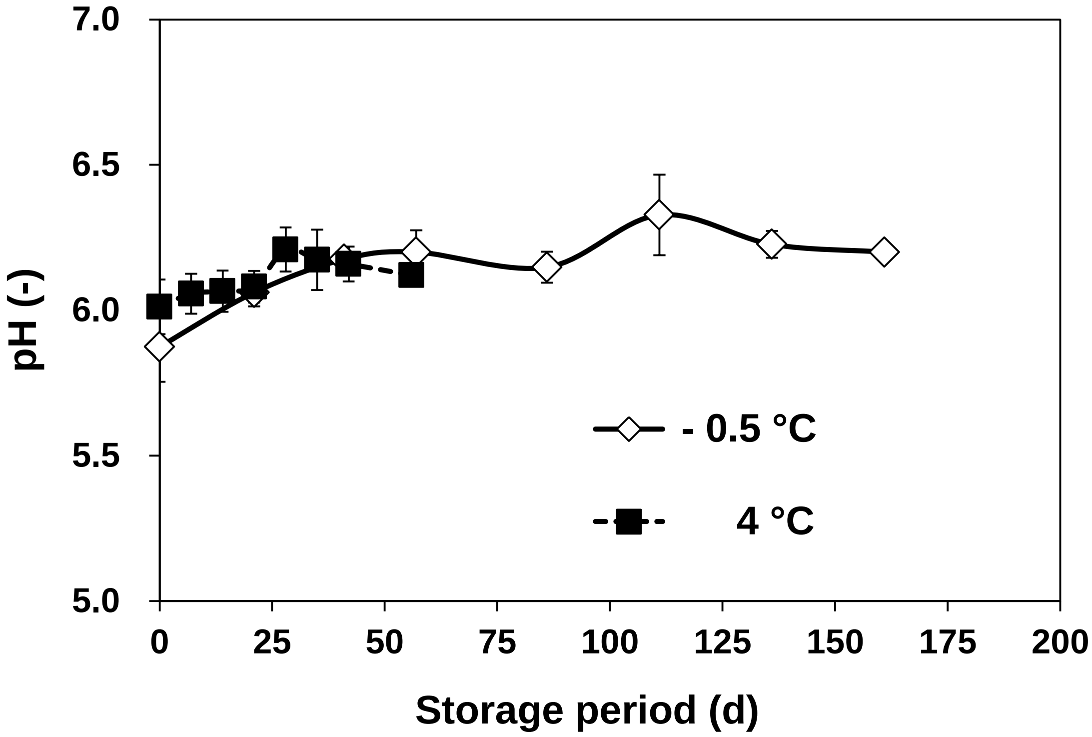


Fig. 4-6 pH value variation during storage period. Error bars indicate standard error (n=3)

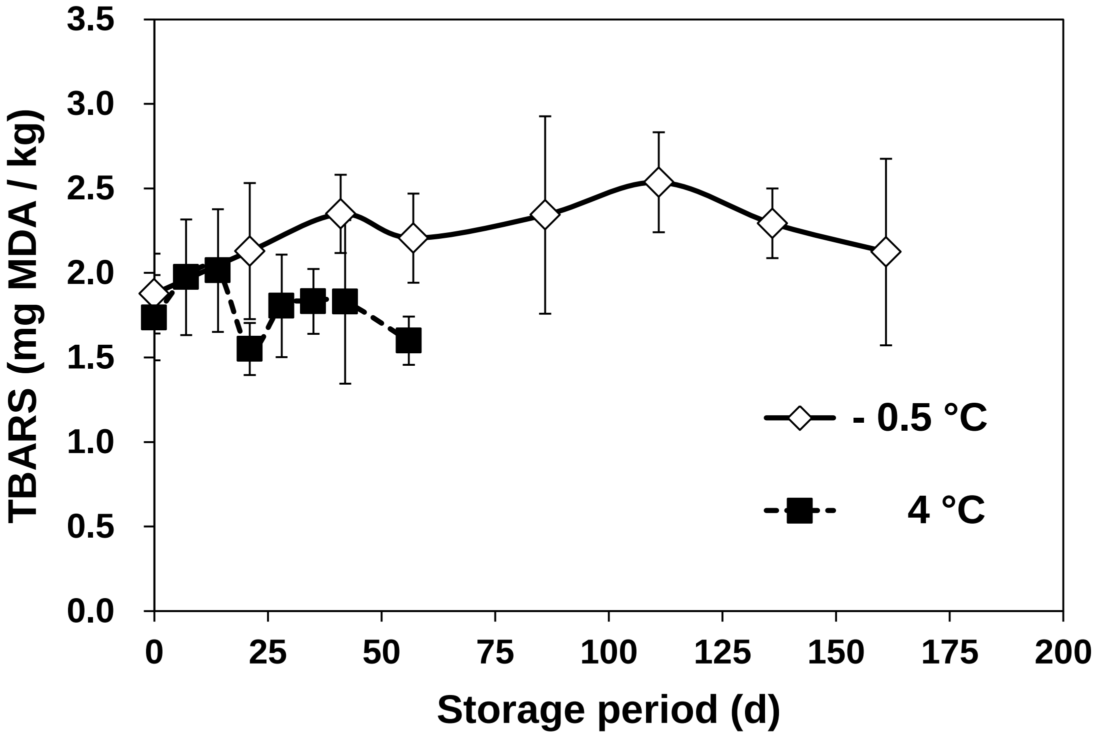


Fig. 4-7 TBARS(2-thiobarbituric acid reactive substance) variation during storage period. Error bars indicate standard error (n=3)

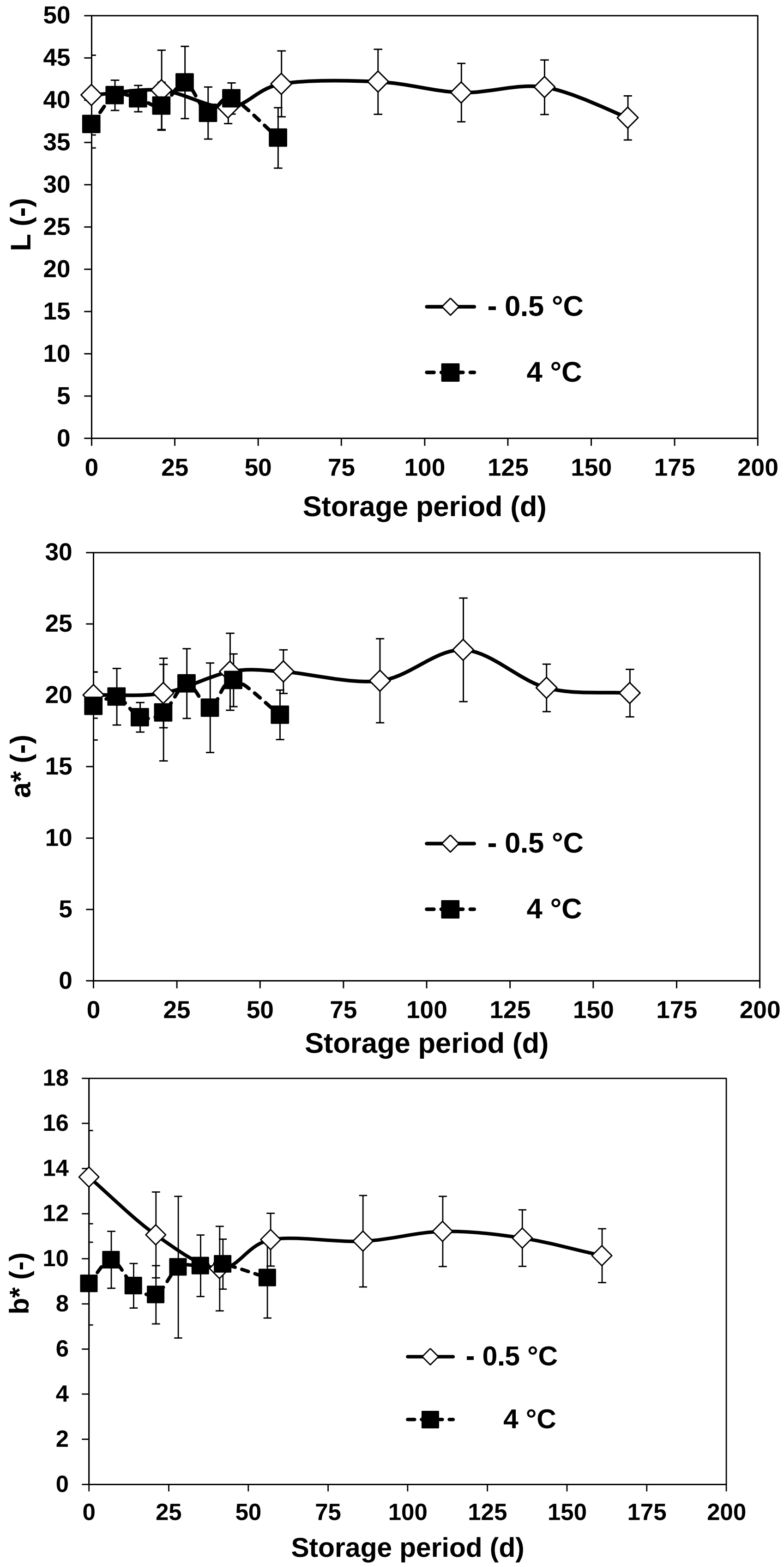


Fig. 4-8 Color variations during storage period. Error bars indicate standard error (n=3)

4.3.4. Microbiological analysis

The aerobic plate counts (APC) of vacuum-packaging beef increased by almost 7.00 log CFU/cm^2 for 85 days storage at -0.5℃ (6.95 log CFU/cm^2) and for 21 days storage at 4℃ (Fig. 4-9). The APC of 7.00 log CFU/cm^2 is supposed to be occurrence of odor (Meat and livestock Australia 2014) and is used the criterion for microbiological management (All Japan Meat Industry Cooperative Associations 2015; Japanese Consumers Co-operative Union 2016). Therefore, storage for 85 days at -0.5℃ and for 21 days at 4℃ were considered to be storage limits for vacuum-packaging beef.

The maximum population density of the APC during storage was around 8.00 log CFU/cm^2. This result was agrees with previous studies on vacuum-packaging beef such as Seideman and Durland (1983), Bożena et al. (2011), Ercolini et al. (2011), and Hernández-Macedo et al. (2011).

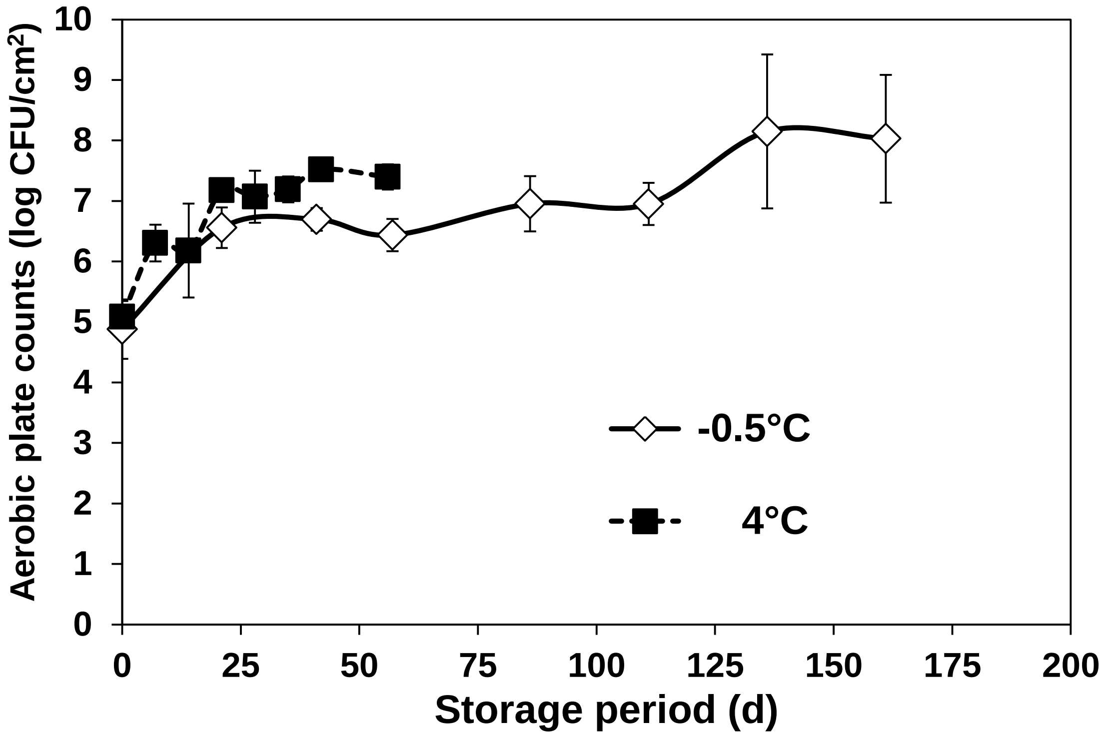


Fig. 4-9 The microbiological variation during storage period. Error bars indicate standard error (n=3)

4.3.5. Shear force

Shear force of beef decreased as the storage period prolonged, in particular the decrease was remarkable at higher (4℃) storage temperature (Fig. 4-10). The decrease of shear force was also reported by Lee and Yoon (2001) and Moon (2005). The decrease would be attributed by moisture contents, degeneration of protein or lipid (Park et al. 2016).

However, the shear force would be an inappropriate criterion for determining shelf-life, because there might be a possibility of decrease in shear force even after spoilage. In fact, the decomposed sample stored at -0.5℃ for 118 days did not show any irregular value of shear force unlike the drip loss, pH, and APC. In addition, the shear force of the samples stored at 4℃ continuously decreased without particular trend.

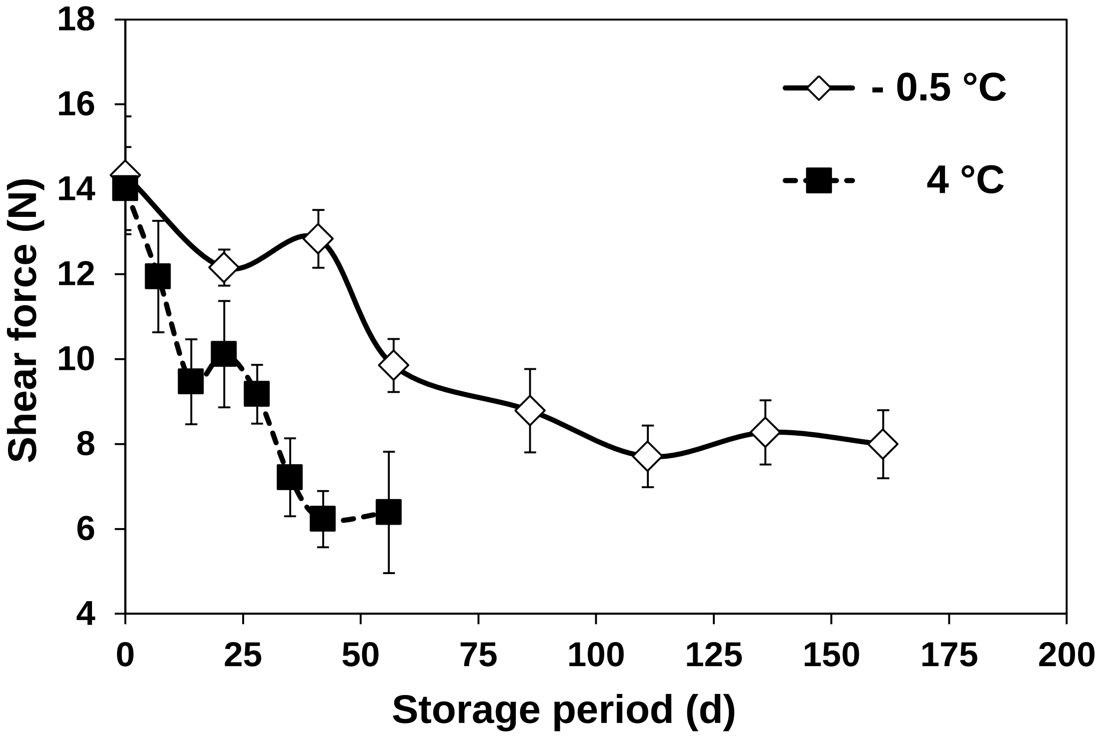


Fig. 4-10 The shear force variation during storage period. Error bars indicate standard error (n=3)

4.3.6. Cooking loss

Cooking loss of beef represented the amount of decrease of meat juice during cooking process at 230℃. Although the beef stored at -0.5℃ did not show significant changes in cooking loss during 21-96 days($p = 0.648$), the amount of cooking loss rapidly increased after 118 days storage. Furthermore, the cooking loss of the beef stored at 4℃ rapidly increased from 17.08 % to 22.52 %after 21 days storage (Fig. 4-11). Bhattacharya and Hanna (1989), Farouk et al. (2004), and Ko and Yang (2001) also reported that the cooking loss increased as the storage period prolonged. The cause of increase in cooking loss would be related to the decrease in water holding capacity of beef muscle in common with the phenomena of drip loss. Rapid increase in the cooking loss appeared on 111 days at -0.5℃ and 21 days at 4℃ might be attributed to protein denaturation induced by spoilage with bacterial growth.

The trend of the changes in drip loss, APC, and cooking loss was similar as such the maximum value appeared on 111 days storage at -0.5℃ and on 21 days storage at 4℃. Therefore, these storage periods at each temperature would be considered as a criterion of shelf life of beef, which will be corresponded to color change in TTI for the vacuum-packaging beef

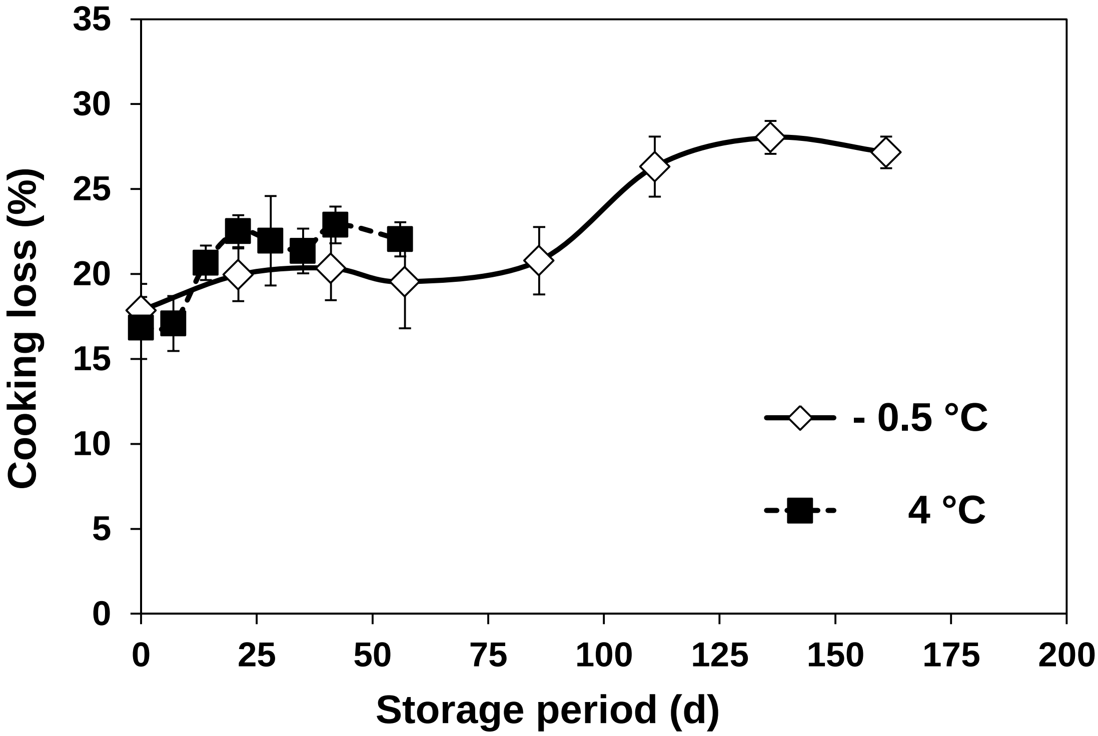


Fig. 4-11 Cooking loss variation during storage period. Error bars indicate standard error (n=3)

4.3.7. Sensory evaluation

The results of sensory evaluation were shown in Fig. 4-12 (a)-(d). The most important aspect of the sensory evaluation for judgement of the shelf life of beef is score of odor and appearance. Off-odors and discoloration are typical criterion for decision of food spoilage or shelf-life (Blixt and Borch 1999). The both odor and appearance score of the beef stored at -0.5℃ rapidly decreased after 86 days storage. Moreover, the odor and appearance score of the beef stored at 4℃ decreased after 21days storage. Thus, the storage at -0.5℃ for 111 days and the storage at 4℃ for 21 days would be considered as a criterion of shelf-life for vacuum-packaging beef.

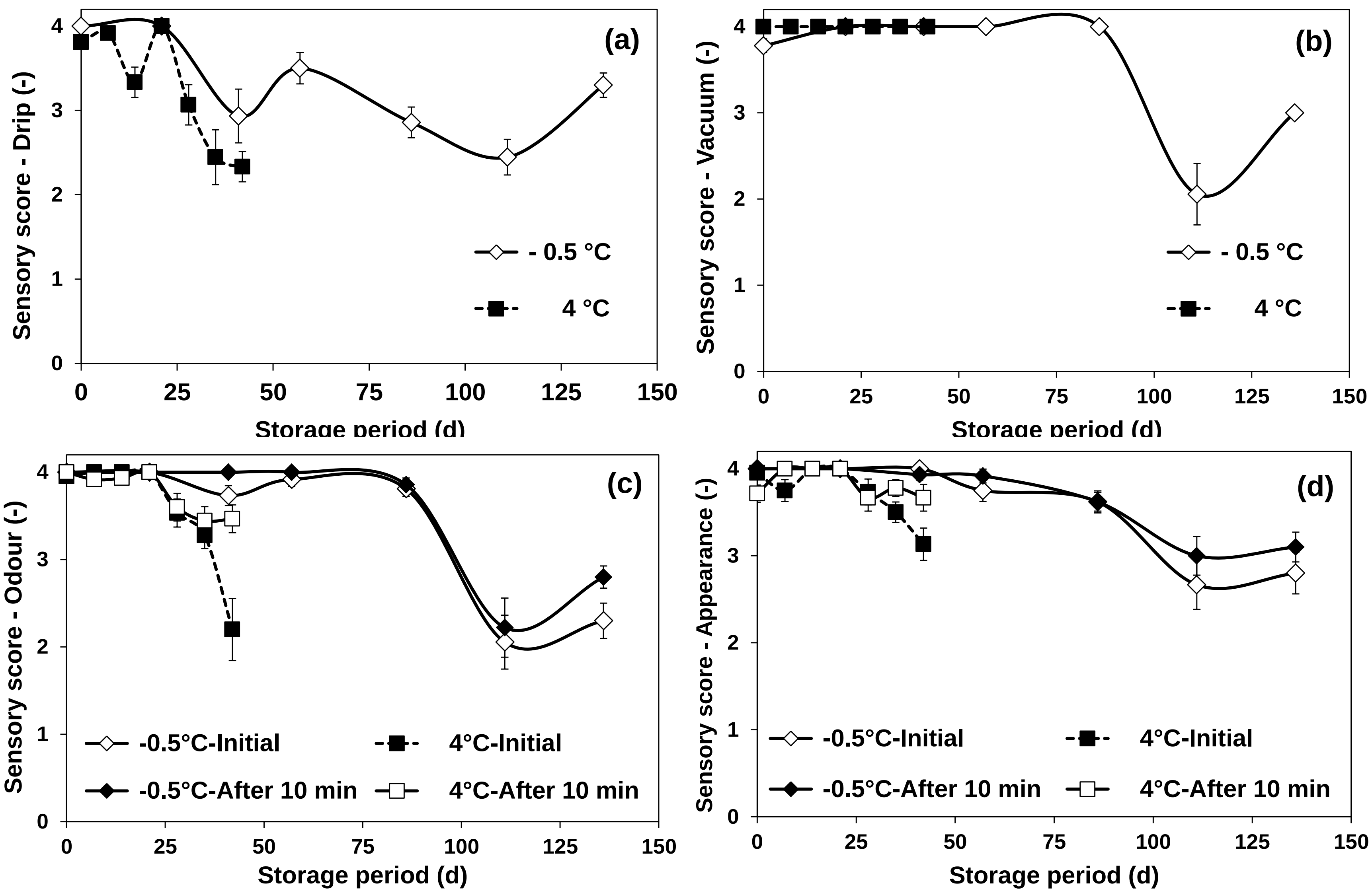


Fig. 4-12 Sensory score variation during storage period. Error bars indicate standard error (n=3)

4.3.8. Application of TTI

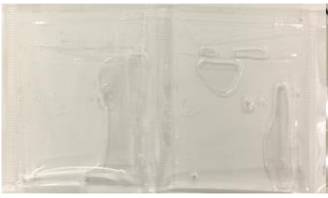
The quality of vacuum packing beef stored at -0.5℃ and 4℃ significantly changed for 86-111 days and 21-28 days, respectively, as described in the previous sections. Based on these results, we assumed that the shelf-life of beef with vacuum-packaging was 111 days at -0.5℃ (150 days including shipping period) and 28 days at 4℃ (68 days including shipping period).

Meanwhile, the changes in color of the examined TTI appropriately reflected the shelf-life of beef with vacuum-packaging (Table 4-5). The brown and yellow color of the examined TTI for stored beef at -0.5℃ was clearly appeared on 111 days storage. Moreover, the color of the examined TTI for stored beef at 4℃ was clearly appeared on 28 days storage. The changes in the TTI's colors from colorless, light blue, to light brown appeared before 111 and 28 days storage at -0.5℃ and 4℃, respectively. These color change allows to estimate the safety of vacuum-packaging beef or to confirm and predict the shelf-life of the beef. Furthermore, the color such as dark brown or dark yellow appeared after 111 or 28 days storage would enable to indicate the quality degradation or the spoilage of beef.

However, the concentrations of reactant in the examined TTI would be needed to adjust for more suitable color change for the shelf-life of vacuum-packaging beef, because the examined TTIs in this study were developed on the basis of on 77 days for 0℃ or 30 days for 5℃, which are current guidelines for beef distribution.

The examined TTIs did not provide exact remaining shelf-life and quality degradation level, because the TTIs showed just only the color. To recognize remaining shelf-life and quality degradation level from the color change of TTI, a dedicated method or tool for color recognition will be necessary for offering various information.

Table 4-5 The color variation in a made TTI (Time-Temperature indicator)

-0.5 ℃			
	21 days	41 days	57 days
			
	86 days	111 days	161 days
4 ℃			
	7 days	14 days	21 days
			
	28 days	35 days	42 days

4.4. Conclusion

The relationship between the quality change of vacuum-packaged beef during shipping and storage and the color change in the developed TTI was investigated for clarifying the availability of the examined TTI for a real shipping and storage site of vacuum-packaged beef.

The TTIs examined for shipping appeared light yellow when arrival. The light yellow indicated that the temperatures during shipping were overall constant with sufficiently low. In addition, the color of the examined TTIs appropriately indicated for the shelf-life of the stored beef with vacuum-packaging. However, the TTI can be improved for more suitable color change corresponding to the shelf-life of vacuum-packaging beef.

Chapter 5 Application of a developed TTI for visualization of heating state of processed foods

5.1. Introduction

Most consumers are demanding safer and higher quality raw and processed foods. To correspond to the consumers' demands and also complicated distribution chain, food processors and distributors are focusing on quantitative monitoring and controlling critical points throughout food chain. In response to such demands, food industry began to seek solutions through an effective food safety management system (FSMS) that is based on controlling, monitoring, and recording the critical parameters (Lim et al. 2014; Nychas et al. 2016). One of the most successful representative system is Hazard Analysis and Critical Control Points (HACCP) (Kafetzopoulos et al. 2013; Khandke and Mayes 1998). In addition, concepts of the appropriate level of protection (ALOP) and the food safety objective (FSO) have been proposed (FAO/WHO 2014; ICMSF 2002) to establish a link between public health outcomes and metrics in the food chain (Koutsoumanis and Gougouli 2015). Although systems for ensuring food safety mentioned above have been proposed so far, there are occasional failures to prevent microbial and/or chemical contamination of food products, which leads to reduced confidence among consumers (Nychas et al. 2016).

Escherichia coli O157:H7 outbreaks have mainly been associated with the consumption of undercooked ground beef patties (Rhee et al. 2003; Nair et al. 2016). Since *E. coli* O157:H7 is not highly thermal resistant, certain cooking to meet a recommend internal temperature would be able to ensure the safety of food product. However, attainment of the recommended cooking temperature might be difficult. For example, Bogard et al. (2013) reported that a majority (77%) of managers of the 385 restaurants did not always measure the final cook temperatures of hamburgers with a thermometer, and twelve percent of the hamburgers were undercooked (cooked to 68.3℃). A survey by the United States Department of Agriculture (USDA) on hamburger cooking practices revealed that 20% of the participants cooked patties rare or medium rare (USDA 2002), which could result in the survival of *E. coli* O157:H7 (Nair et al. 2016). The use of thermometers to determine meat temperature during cooking of beef patties has been limited (National Cattlemen's Beef Association, 1999) due to the inconvenience and uncertainly about the value of the procedure USDA 2002). Even though predictive microbiology models describing thermal death behaviors of pathogenic microorganisms in foods could make easier setting of thermal cooking condition, proper thermal management is not necessarily performed based on the predictive model prediction. One of the reason would be due to lack of appropriate temperature monitoring procedure. Thus, a development of hardware has been needed to simply and visually monitor temperature history during thermal processing (Koutsoumanis and Gougouli, 2015).

Time temperature integrators/indicators (TTIs) are one of the promising technologies for monitoring temperature history of food products (Koutsoumanis and Gougouli 2015). Thus, we designed the present study to develop a TTI for monitoring thermal management during cooking. Japanese Ministry of Health, Labour and Welfare (JMHLW) have been proposed that a core temperature of meat products at least 75 ℃ is reached and holding for more than 1 min. In the present study, we targeted this criterion for evaluation of thermal processing. To develop TTIs for monitoring thermal management during cooking, following problems should be solved; (1) TTIs are applicable for the various temperature conditions and rising temperature. (2) The changes in color of TTIs can be easily evaluated. (3) Predictions of changes in color of TTIs can be obtained both at constant and fluctuating temperature.

Maillard-reaction based TTI would have a possibility to solve the above problems. For temperature management during cooking, Maillard-reaction based TTI has following some advantages;

・Consumer-friendly tool to monitor

・No need to keep the TTI cool before use (Rokugawa and Fujikawa, 2015)

・Adjustable the kinetics of the color change by changing the concentrations of both the reducing sugar and amino acid solutions for the TTI

・Enabling to predict the changes in color by kinetic models

The objective of this study was to develop a Maillard-reaction based TTI for monitoring temperature history during cooking. In particular, to ensure sufficient heating of internal temperature of a meat product such as hamburger, we targeted a criterion of 75℃ for more than 1 min suggested by JMHLW.

We investigated conditions of Maillard reaction which show obvious color change at 75℃ for 1 min in water bath. In hamburgers, the color variation of TTI was evaluated in a real heating process. In addition, the changes in color were analyzed quantitatively and developed a predictive model for color changes as a function of temperature and time. The performance of the developed models was validated under fluctuating temperature conditions in water bath and also in hamburgers.

5.2. Materials and methods

5.2.1. Chemicals for Maillard reaction

As per Yamamoto and Isshiki (2012) and Laroque et al. (2008), we used combinations of D-xylose and glycine (both from Nacalai Tesque, Inc., Kyoto, Japan) or D-ribose and L-lysine (both from Wako Pure Chemical Industries, Ltd., Osaka, Japan), respectively, to create highly reactive Maillard reaction systems and investigated the effects of the concentration of both the reducing sugar (1.0 to 7.0 M) and the amino acid (1.0 to 3.0 M) on the color variation of the reaction. In addition, 0.5 M dipotassium phosphate (K_2HPO_4; Kanto Chemical Co., Inc., Tokyo, Japan) was used to achieve a suitable reaction rate. These concentrations were based on the solubility of each reagent and the aforementioned studies.

5.2.2. Changes in color by Maillard reaction under heating conditions

The solution of the reducing sugar and the solution containing the amino acid and K_2HPO_4 were placed in separate glass tubes and heated in a water bath consisting of a styrofoam box (240 mm × 156 mm × 170 mm) and a heater (Thermo-Mate BF400, Yamato Scientific Co., Ltd., Tokyo Japan). Aliquots of the solutions (500 μL of each) were pipetted and mixed in another glass test tube placed in the water bath, thus initiating the Maillard reaction at the set temperature.

5.2.3. Monitoring of the color variation of the Maillard reaction at constant temperatures

To establish the temperature dependency of the color variation in the Maillard reaction, we examined changes in color under isothermal conditions at various temperatures (45, 55, 65, 75, 85, and 90℃). Color variations were recorded by automatically taking pictures at intervals of 2 s using the built-in digital camera (resolution, 1920 × 1080 pixels; aperture, f/2.2; focal length, 4.00 mm) of an iPhone 5s (Apple, Inc., Cupertino, CA, USA) that had been fitted on the lid of the water bath. Use of the lid was necessary in order to block off environmental light. The camera was set at optimized exposure conditions to obtain the best determination performances, i.e., automatic white balance and automatic sensitivity (ISO speed, 160).

5.2.4. Analysis and prediction of color variation

Color variations recorded by the digital camera were analyzed and expressed in the RGB color system for objective assessment. It is easy to obtain RGB data from digital pictures, avoiding the use of color-measuring instruments such as $L^*a^*b^*$ colorimeters. Image analysis was performed using the R statistical environment (v3.2.0) running in R studio (v1.0.44). Color variation (Red, Green, or Blue value) at a constant temperature was described as a modified logistic equation as follows:

$$C_v\,[R,G,B] = -\frac{\Delta C_v}{1+\exp(-\mu_{\max}(t-T_{\inf}))} \qquad \text{(Eq.5-1)}$$

where C_v is the color in the RGB system (R, G, or B value), ΔC_v is the difference between the maximum and the minimum value of C_v, μ_{max} is the maximum change rate (of R, G, or B), t is time elapsed (s), and T_{inf} is the time of the inflection point of the kinetics (s).

Each parameter obtained by fitting the data of the process to Eq. 5-1 was described by multiple regression analysis as a function of the concentration of the reactants and the reaction temperature as follows:

$$Paramater[\Delta C_v, T_{inf}, \mu_{max}]$$
$$= a_0 + a_1 \times Temp + a_2 \times Conc + a_3 \times Temp \times Conc \quad \text{(Eq.5-2)}$$

where *Conc* is the concentration of the reactants and Temp is the reaction temperature. Accordingly, changes in C_v at fluctuating temperature, which must be taken into account when considering temperature history, can be numerically calculated by substituting the corresponding parameter in Eq. 5-1 with Eq. 5-2.

5.2.5. Color variation in Maillard reaction under rising temperature conditions

To confirm the predictions of the mathematical model regarding the color changes of a Maillard reaction in a real heating process, the color variation was monitored during heating at rising temperature (20–90℃), using the equipment described in section 5.2.3. The temperature in a test tube containing both the reducing sugar and the amino acid was monitored by using a type K thermocouple connected to a data logger (Multichannel Recorder MCR-4TC; T&D Corporation, Nagano, Japan). Color variations were analyzed as described in section 5.2.4.

5.2.6. Practical application of Time-temperature indicator (TTI) in hamburger cooking

In order for a TTI to be suitable for thermal monitoring during cooking, its color changes should take place on a time scale relevant to the cooking process. Therefore, the time necessary for switching between TTI color variations was determined in an actual hamburger cooking process. All the ingredients used to prepare the patties (Table 5-1) were purchased from a local retailer. They were placed in a sterile stainless bowl, where they were folded 50 times and mixed 300 times using a rubber spatula. Afterward, the mix was divided into 100-g portions, which were shaped into patties (ca. 2.0 cm in thickness, 8 cm in diameter).

Table 5-1 Composition of examined hamburger

Ingredient	Weight (g)
Ground beef and pork	200.00
Chopped onion	100.00
Milk	20.00
Egg	20.00
Breadcrumbs	20.00
Salt	2.00
Pepper	0.02

The TTI examined in this study was based on the principles of the Maillard-type TTI developed by Yamamoto and Isshiki (2012) and consisted of two sterile transparent pouches (both 15 mm × 25 mm × 1 mm), one of which (A) contained 100 μL of the d-ribose solution, while the other (B) had 100 μL of the l-lysine/K2HPO4 solution. All the solution was filter-sterilized (pore size 0.22 μm) before placing into the pouch. The reaction was initiated by manually breaking the seal between the pouches.

Patties were held at 20 ℃ in an incubator. A type K thermocouple (connected with MCR-4TC Multichannel Recorder) and the Maillard-type TTI were inserted into the center of the hamburger patty, and the patties were cooked individually in a steam convection oven (HEALSIO AX-CX3; SHARP, Tokyo, Japan) at 230 ℃. The center of the hamburgers was heated at 75 ℃ for 1 min. Immediately after the 1-min stand, the color variation of the indicator was observed with the naked eye and photographed with the digital camera.

5.2.7. Thermal inactivation of Escherichia coli O157:H7 in hamburgers

We used four strains of E. coli O157:H7 (RIMD 0509939, RIMD 05091896, RIMD 05091897, and HIPH 12361) in this study that were kindly provided by the Research Institute for Microbial Diseases (RIMD) of Osaka University and the Hokkaido Institute of Public Health (HIPH). The strains were maintained at − 80 °C in tryptic soy broth (TSB) containing 10% glycerol. A platinum loop was used to streak bacteria from the frozen bacterial cultures on tryptic soy agar (TSA) plates. The inoculated plates were incubated at 37 ℃ for 24 h, and then, an isolated colony of each bacterium was transferred to fresh 5 ml TSB in a sterile plastic tube. The cultures were transferred using loop inoculation through two successive 24-h intervals to obtain more homogeneous and stable cell populations. Grown cells were collected by centrifugation (3000×g, 10 min), and the resulting pellets were washed with sterile 0.1% peptone water to remove traces of the TSB medium. After the wash, bacteria were resuspended in peptone water to a concentration of 8~9 log CFU/mL. The resuspensions of the four strains were mixed to prepare culture cocktails that were used for inoculations.

Hamburger samples were prepared as described in section 2.6 and divided into 300-g batches. Each batch was inoculated with 3 mL of E. coli O157:H7 cocktail, resulting in a bacterial density of 6~7 CFU/g, mixed 150 times using a rubber spatula in a sterile stainless bowl, and then divided into three hamburger patties, which were cooked as described in section 2.6. Immediately after the 1-min hold at the target internal temperature of 75 ℃, hamburgers were immediately placed into sterile plastic bags and plunged into a water/ice mixture to cool. Afterward, they were divided into 25-g samples that were suspended in 225 mL of 0.1% peptone water and homogenized by a stomacher (Stomacher 400-T; Seward, Worthing, UK) for 60 s. Serial 10-fold dilutions of the suspended samples were prepared in 0.1% peptone water, and 100-μL aliquots were spread on duplicate (TSA) plates, which were incubated at 37 ℃ for 24 h and then examined for the presence of colonies. The experiment was conducted in triplicate.

5.3. Results and discussion

5.3.1. Selection of Maillard reaction substrates

Measuring the internal temperature of a food product is not sufficient for evaluating the effectiveness of a thermal cooking process, as it does not guarantee that the product remained at an adequately high temperature for sufficient time to ensure safe consumption. Thus, we need a procedure that not only monitors temperature but also indicates temperature history during cooking. A TTI showing an easily measurable, time- and temperature-dependent change reflecting the full or partial temperature history of a food product would be ideal for the thermal monitoring of the cooking process (Koutsoumanis and Gougouli 2015). As mentioned above, clear color change when heated at 75 ℃ for 1 min was set as the criterion for choosing a Maillard reaction system for use in the evaluation of food thermal processing. Fig. 5-1 shows the temporal changes in the color of a reaction system consisting of 7.0 M D-xylose, 3.0 M glycine, and 0.5 M K_2HPO_4. As the color of the solution did not display considerable change after 1 min at 75 ℃ (the change was not very intense even after 2 min), we abandoned D-xylose/glycine reaction systems as a potential base for our TTI.

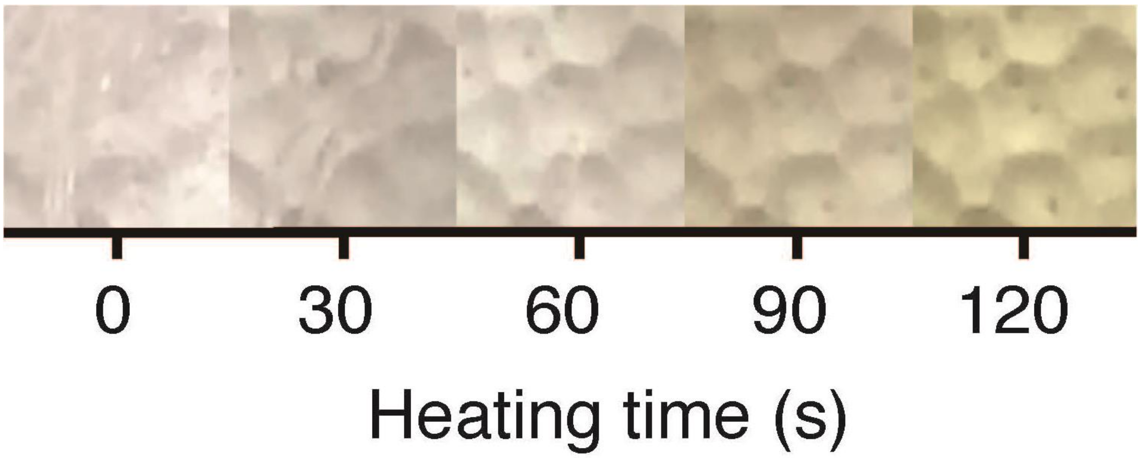


Fig. 5-1 Temporal color changes in a Maillard reaction system containing 7.0 M D-xylose, 3.0 M glycine, and 0.5 M K_2HPO_4, placed in a water bath at 75 ℃

We repeated the test with a Maillard reaction system consisting of 7.0 M D-ribose, 3.0 M L-lysine, and 0.5 M K_2HPO_4. As seen in Fig. 5-2, the color of the solution displayed obvious changes after 1 min at 75 ℃. For this reason, D-ribose/L-lysine systems were chosen for further analysis, namely the evaluation of the most suitable combination of substrate concentrations. First, we examined the effect of the concentration of D-ribose (1.0–7.0 M) on the reaction time it takes for the solution to turn brown (R < 0.6, G < 0.3, and B < 0.1 in the RGB color system) at 75 ℃, in the presence of 3.0 M L-lysine and 0.5 M K_2HPO_4. As shown in Fig. 5-3, increasing the concentration of D-ribose above 3.0 M did not lead to considerable higher reaction rates; thus, this concentration of ribose was adopted.

Next, we examined the effect of various concentrations of L-lysine (1–3 mg/kg) on the temporal changes in color observed at 75 ℃ in the presence of 3.0 M D-ribose and 0.5 M K_2HPO_4. As shown in Fig. 5-4, the changes in color were L-lysine concentration-dependent, with 3.0 M L-lysine giving the clearest color differences under the specified conditions. Therefore, we decided to adopt a Maillard reaction system of 3.0 M D-ribose, 3.0 M L-lysine, and 0.5 M K_2HPO_4, as it provides clear visual information for the achieving the desired thermal condition.

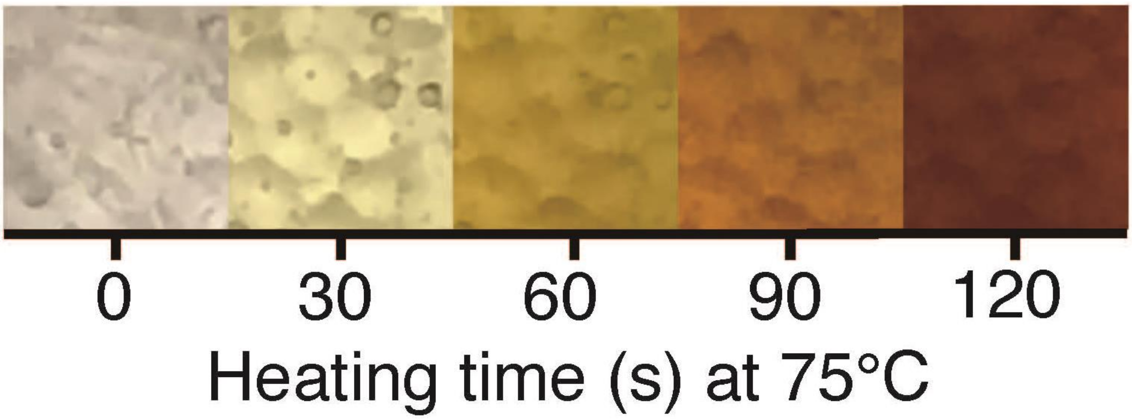


Fig. 5-2 Temporal changes of color in a Maillard reaction system containing 7.0 M D-ribose, 3.0 M L-lysine, and 0.5 M K_2HPO_4, placed in a water bath at 75 °C

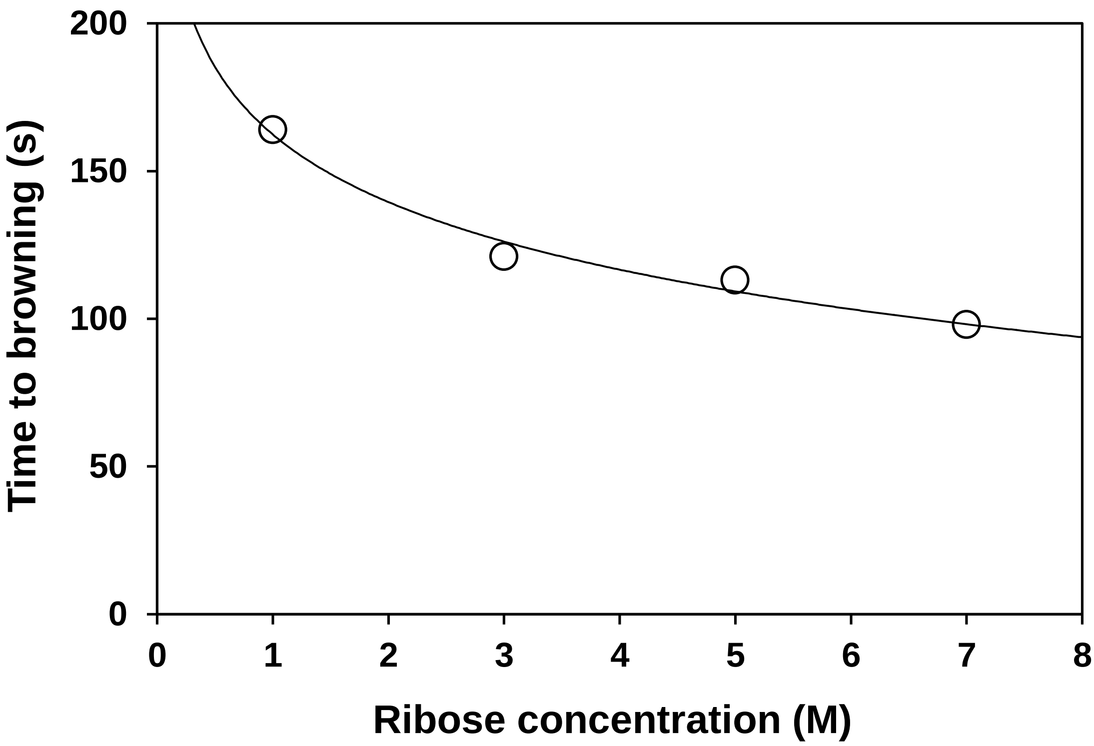


Fig. 5-3 Effect of D-ribose concentration (1.0–7.0 M) on the reaction time needed for a solution containing 3.0 M L-lysine and 0.5 M K_2HPO_4 to turn brown ($R < 0.6$, $G < 0.3$, and $B < 0.1$ in the RGB color system) at 75 °C

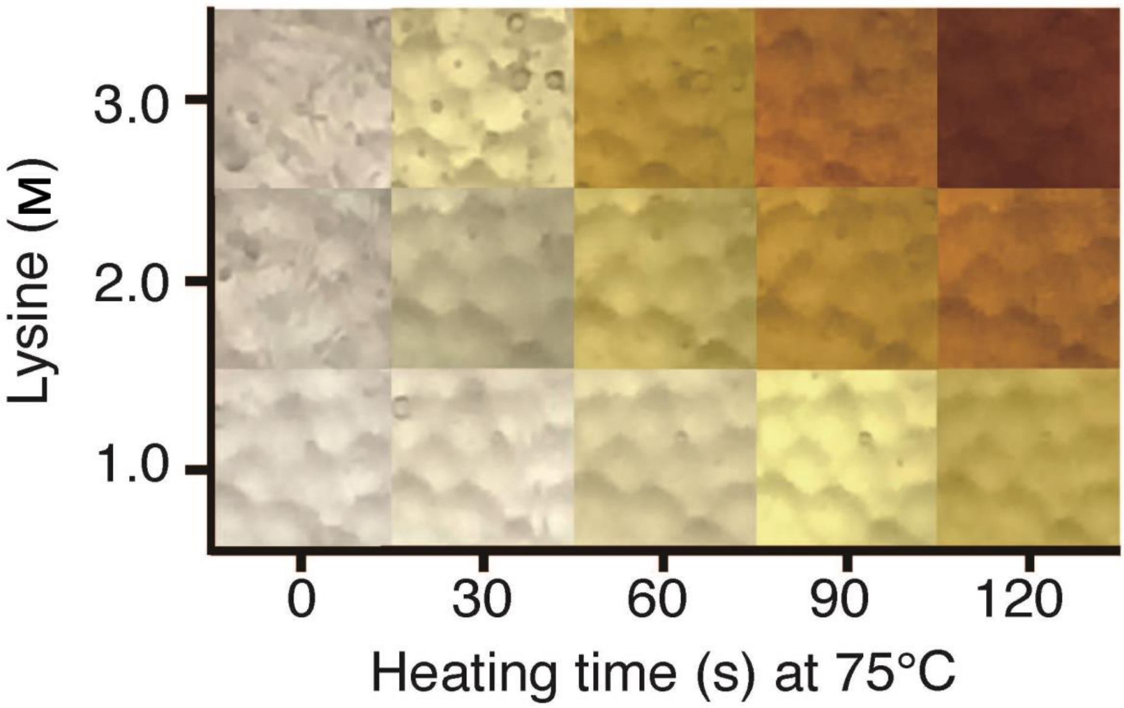


Fig. 5-4 Temporal changes of color in Maillard reaction systems containing 3.0 M D-ribose, 1.0–3.0 M L-lysine, 0.5 M K_2HPO_4, and various concentrations of lysine (1.0–3.0 M) at 75 °C

5.3.2. Color change kinetics analysis

We performed a kinetics analysis of the color displayed by the adopted Maillard reaction system (3.0 M D-ribose, 3.0 M L-lysine, and 0.5 M K_2HPO_4) when heated at a constant temperature by taking digital photographs and using them to express the colors in the RGB system. The tested temperatures were 45, 55, 65, 75, 85, and 90 ℃. The obtained color variations at all temperatures were accurately described by a modified logistic equation (Eq. 5-1), regardless of the tested conditions, as indicated by the low value (< 0.06) of the small root mean squared error (RMSE). Since the fitted parameters, T_{inf}, and μ_{max} changed exponentially with temperature, their logarithmic values were used for multiple regression analysis as described in Eq. 5-2. The multiple regression parameters for T_{inf} and μ_{max} are summarized in Table 5-2. The logarithmic values of both T_{inf} and μ_{max} had a high goodness-of-fit under the tested temperature conditions, as indicated by their R^2 values (> 0.98). The fitted parameter (ΔC_v) was almost constant at these temperatures, with an average value of 0.75 ± 0.03. Replacing the parameters in Eq. 5-1 with Eq. 5-2 allows describing the color as a function of temperature and reagent concentration.

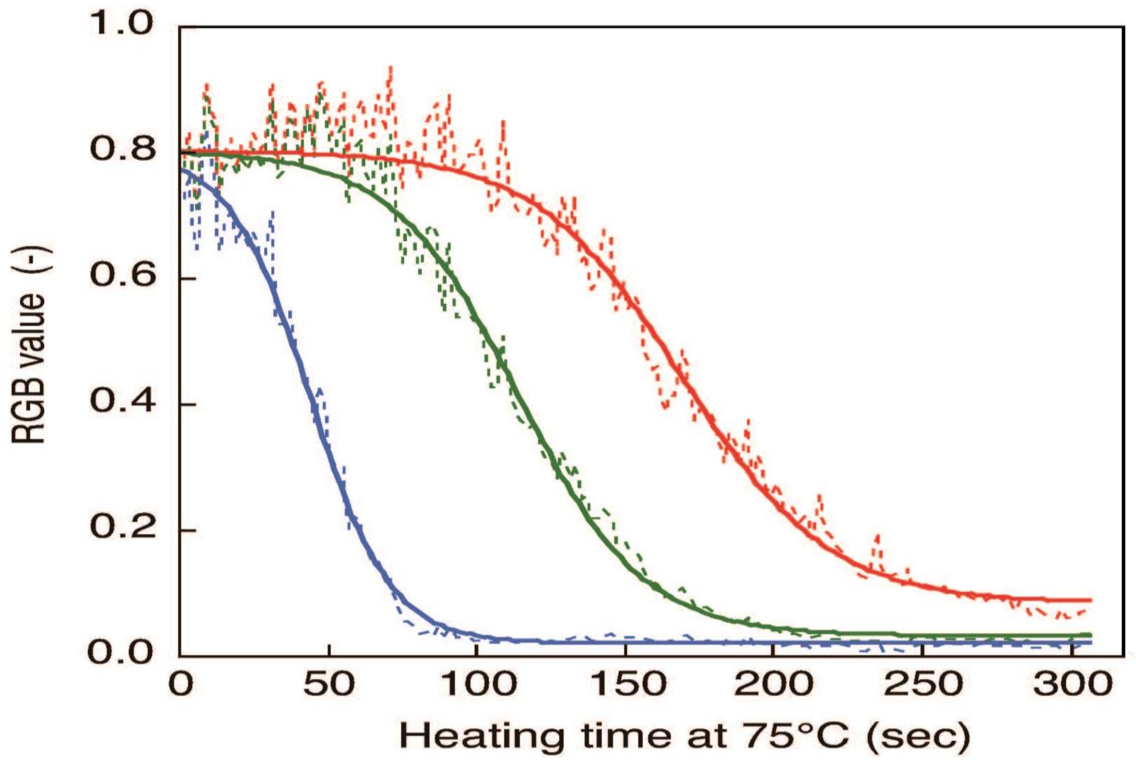


Fig. 5-5 A representative plot of the predicted (solid lines) and the observed (dashed lines) temporal changes in the R, G, and B values (colored red, green, and blue, respectively) of a Maillard reaction system containing 7.0 M D-ribose, 3.0 M L-lysine, and 0.5 M K_2HPO_4 at a constant temperature of 75 °C

Table 5-2 Estimated model parameters for the logarithms of μ_{max} and T_{inf} in the modified logistic equation describing the color variation of a Maillard reaction system consisting of 7.0 M D-ribose, 3.0 M L-lysine, and 0.5 M K_2HPO_4 at constant temperature (45–90 °C). R^2, correlation coefficient

Color	Parameter	Coefficients				R^2
		a_0	a_1	a_2	a_3	
R	μ_{max}	-12.2672	0.091844	0.356467	0.004103	0.990261
	T_{inf}	13.97426	-0.09929	-0.476	-0.0000865	0.99616
G	μ_{max}	-12.1821	0.099285	0.48146	0.000472	0.989732
	T_{inf}	13.54573	-0.10227	-0.4955	0.001048	0.994449
B	μ_{max}	-11.8926	0.106592	0.721061	-0.00395	0.991147
	T_{inf}	12.23882	-0.09626	-0.44248	-0.00013	0.994835

5.3.3. Experimental validation of the developed model

We tested the accuracy of the developed model in predicting temporal color changes. Figure 5-6 shows the observed and the predicted color changes of a Maillard reaction performed at rising temperature in test tubes, both expressed as RGB values. Overall, there was a good agreement between the predicted and the observed RGB values (RMSE < 0.009). We conclude that the developed model can indeed predict the changes in the color of a Maillard reaction performed under arbitrary heating conditions.

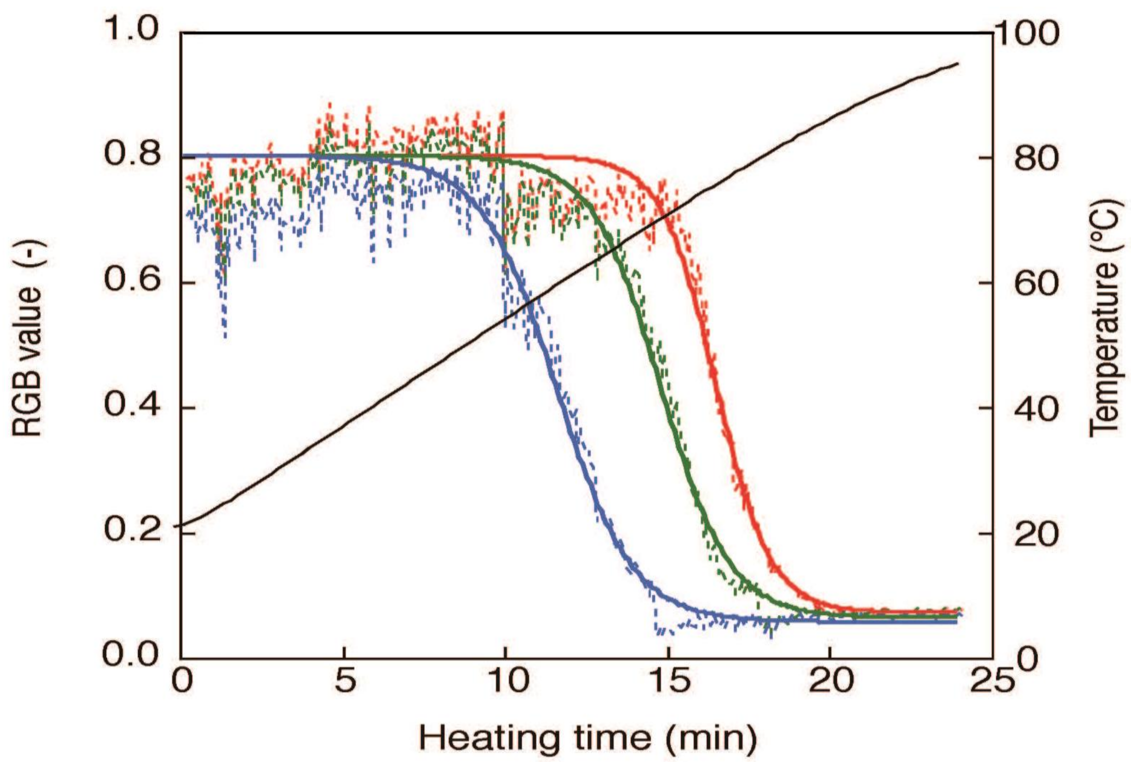


Fig. 5-6 A plot of the predicted (solid lines) and the observed (dashed lines) reductions in the R, G, and B values (colored red, green, and blue, respectively) in a Maillard reaction system containing 7.0 M D-ribose, 3.0 M L-lysine, and 0.5 M K_2HPO_4, placed in a water bath at rising temperature (20 to 90 °C). The black solid line shows the temperature history during the heating process

Using a real heating process, we demonstrated that the color change of the Maillard reaction in a D-ribose/L-lysine system provides clear visual information for evaluating thermal processes. By optimizing various parameters that are known to affect the Maillard reaction, such as the type and concentrations of sugar and amino acid (Laroque et al. 2008), the pH (Martins et al. 2000), and metal ions (Ramonaitytė et al. 2009), we developed a TTI based on a D-ribose/L-lysine Maillard reaction system for the evaluation of thermal processes.

5.3.4. Performance of the Maillard eaction as a TTI for hamburger cooking

To validate the performance of the Maillard reaction-based TTI for the thermal evaluation of an actual cooking process, we examined its color change while cooking hamburgers. The TTI satisfied the set criterion, as its color clearly changed from light yellow to brown during the 1-min hold at 75 ℃ (Fig. 5-7). Thus, our Maillard-based TTI can successfully provide visual information for evaluation of thermal processes. In addition to observation with the naked eye, we analyzed the digital photographs of the TTI shown in Fig. 5-7 to quantitatively evaluate the color changes of the TTI in the hamburger during the cooking process. By the time that the internal temperature of the hamburger reached the target temperature of 75 ℃, no significant changes in the color of the TTI had yet been observed, either with the naked eye or by analysis of the RGB values from the digital pictures (changes in RGB values were lower than 0.04). In contrast, color changed significantly after 1 min at 75 ℃ (R: 0.18, G: 0.40, B: 0.34). Notably, as shown in Table 5-3, there was a significant discrepancy between the predicted and the observed color at the end of the 1-min hold at 75 ℃.

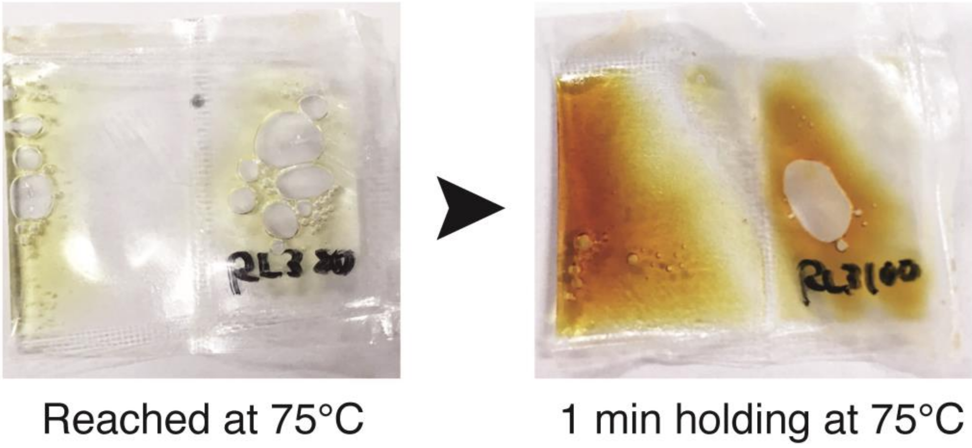


Fig. 5-7 Colors of the Maillard-based TTI at the beginning and the end of the 1-min hold at 75 °C, during a hamburger's cooking process

Table 5-3 Comparison between the RGB values observed in a Maillard-based TTI consisting of 7.0 M D-ribose, 3.0 M L-lysine, and 0.5 M K_2HPO_4 that was placed in the core of a hamburger during the cooking process and those predicted by the developed mathematical model

	Reached at 75℃		After 1 min holding at 75℃	
	Observed	Predicted	Observed	Predicted
R	0.74	0.79	0.60	0.78
G	0.74	0.76	0.34	0.74
B	0.50	0.52	0.04	0.38
Images				

The most important advantage of our TTI is that it enables the user to easily quantify the integrated time-temperature effect without the need for special instruments, as shown in Fig. 5-6. However, measurable values are required for the objective evaluation of color changes. Thus, we used digital photos taken by a smartphone camera to express the color variations in the RGB color system. The kinetics of color changes are usually evaluated in the CIE L*a*b* color space (Mendoza et al., 2006; Vaikousi et al. 2009; Rokugawa and Fujikawa 2015). However, it is difficult to relate L*a*b* values with images observed with the naked eye. Another disadvantage is the need for a dedicated colorimeter. These factors, combined with our objective of developing a smartphone application for evaluating the appropriateness of the cooking process by analyzing digital photos, made us choose the RGB system.

5.3.5. Confirmation of thermal inactivation E. coli O157:H7 in hamburgers

The initial bacterial load of the inoculated hamburgers was 6.09 ± 0.08 log CFU/g. After 1 min at 75 ℃, the bacterial load in all patties, including those that had been inoculated, had fallen below the detectable level (< 1 log CFU/g). With respect to E. *coli* O157:H7 strains, no viable cells were found in any of the cooked patties, even in those that had been inoculated. We conclude that heating the hamburgers at 75 ℃ for 1 min was indeed sufficient for eliminating E. coli O157:H7 (Cassin et al. 1998; Amalaradjou et al. 2010).

The most important characteristic of our TTI is that it does not change color while being heated to 75 ℃, but only after spending 1 min at that temperature. Thus, our system allowed verification of both reaching the targeted temperature and being subjected to it long enough for microbial inactivation to occur. Indeed, E. *coli* O157:H7 in inoculated hamburgers was eliminated after cooking at 75 ℃ for 1 min. This finding indicates that our TTI has the potential of improving the microbiological safety of thermally processed foods.

There was a significant difference between the observed and predicted values of the TTI color in hamburgers (Table 5-3). This may be attributed to (1) errors in modeling and prediction and/or (2) the differences in heat transfer characteristics of water and the patty porousness and materials such as fat and/or protein. However, since the prediction error of our mathematical model was significantly lower than those of the observed values, this error could be considered fail-safe. Although more accurate predictive model will be needed to establish for a commercial application of TTI, the Maillard-based TTI could be applicable in cooking temperature management for ensuring microbial safety.

5.4. Conclusion

All in all, the results of the present study demonstrate that the Maillard reaction-based TTI we developed allows appropriate management and evaluation of temperature history during thermal processing. Moreover, it can be adjusted to various temperature-time combinations by modifying the types of reactants and their concentrations. Importantly, this technology allows managing the thermal cooking process at the individual food product level; hence, it may contribute to achieving more accurate and reliable microbiological control of this process. Further investigation to correspond to the other temperature and holding time conditions should be conducted for various thermal treatments of processed foods in the future. However, the present prototype is impractical as it must be inserted into the center of the hamburger patty. Thus, its design will have to be improved in order for it to become an easy-to-use, commercial product. Further investigation will be aimed at increasing the practicality and availability of the TTI system.

Chapter 6 Summary

Maillard reaction-based TTIs were examined for development of flexible TTIs under various agricultural and food processes such as distribution, storage, ripening and the cooking.

The color variations of Maillard reaction by D-xylose, glycine, and Na2HPO4 were investigated by various conditions of reaction temperatures and reactant concentrations. The color changes were easily recognized by naked eyes. The reaction rate increased with decreasing temperature and reactant concentration. As a consequence, the color variation rate of the Maillard reaction allows the reaction time to be adjusted from 12 h to 1 years by controlling reaction temperature and reactant concentrations. Furthermore, the reaction completion time and color variation could be accurately predicted by the developed mathematical model. Thus, Maillard reaction-based TTIs exhibits higher flexibility of reaction time by adjusting the reaction related parameters.

In addition, the examined TTI developed on the basis of the investigated data was verified the usefulness for distribution, storage, ripening and cooking process. In concrete, the examined TTIs allow to indicate the quality change during storage and ripening process of melon, and appropriately indicate the shelf-life of vacuum packed chilled storage beef. Moreover, the examined TTI demonstrated the applicability for evaluation of sufficiency of thermal cooking processes in various foods.

In conclusion, the studied TTIs based on Maillard reaction exhibited high applicability for various agricultural and food processes. However, there are still issues to be solved for more suitable color change and also the shape of TTI. Furthermore, additional studies will be needed to enhance the usefulness of the TTIs

corresponding to quality change of various foods.

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Development of Maillard reaction based time-temperature indicator/integrator for application of agricultural and food process engineering

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