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# Kinetic modeling and characterization of a diffusion-based timetemperature indicator (TTI) for monitoring microbial quality of nonpasteurized angelica juice



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#### A R T I C L E I N F O

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## ABSTRACT

Time-temperature-indicator, or integrator (TTI), can be used for visual display of food product safety information for consumers. A prototype isopropyl palmitate (IPP) diffusion-based TTI system was characterized and evaluated for monitoring microbial quality of non-pasteurized angelica (NPA) juice based on temperature abuse. Diffusion of IPP in the TTI system was measured at various iso-thermal and dynamic temperatures and a mathematical model based on relationships between diffusion and time-temperature was established. Predicted results from the established model were in good agreement with experimental results. Total aerobe counts in NPA juice reached a critical level of 6 log CFU/mL from an initial load of 3.7 log CFU/mL after 36.6 h and 12.5 h of storage at 15 °C and 25 °C, respectively. IPP diffusion of 7.0 mm and 7.2 mm at 15 °C and 25 °C, respectively. IPP diffusion of 7.0 mm in the TTI system was considered to be a threshold point for bacterial quality of NPA juice. However, the proposed TTI was only verified for indicating temperature abuse above 13.5 °C. The TTI system characterized in this study showed potential for monitoring the microbial quality of perishable food products during distribution and storage.

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# 1. Introduction

Temperature and time, widely recognized as major factors influencing the rate of microbial activity in foods, often deviate from specifications during manufacturing, distribution, handling, and storage (Giannakourou, Koutsoumanis, Nychas, & Taoukis, 2005; McMeekin et al., 2008). Therefore, it is important to monitor changes in temperature and time parameters from production to final consumption to ensure microbial safety and quality of food products (Taoukis & Labuza, 2003). Characteristics of a modern quality and safety assurance system should be based on capability to monitor, record, and control critical parameters to prevent contamination throughout the product life cycle (Lu, Zheng, Lv, & Tang, 2013; Wanihsuksombat, Hongtrakul, &

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### Suppakul, 2010).

A TTI is an intelligent packaging system, usually in the form of a small intelligent tag or label attached to a food product. A TTI indicates the cumulative time-temperature history of a food product using an irreversible chemical change in the TTI device that is detected as a visible response in the form of mechanical deformation, color development, or color movement. A TTI is a simple, costeffective, and consumer friendly device for monitoring, recording, and translating quality information for consumers (Pavelková, 2013; Taoukis & Labuza, 2003; Vaikousi, Biliaderis, & Koutsoumanis, 2008; Zabala, Castán, & Martínez, 2015). The overall effect of the temperature history of a food product on food quality, safety, and shelf-life can be observed using a TTI (Ellouze & Augustin, 2010). Different commercial TTI types have been developed based on enzymatic, polymer, and biological reactions (Ellouze & Augustin, 2010; Kim, Kim, & Lee, 2012; Lu et al., 2013; Wu et al., 2015).

A prerequisite for effective application of a TTI based control

system is kinetic study and modeling of food quality loss indices and of the response of the TTI (Tsironi, Stamatiou, Giannoglou, Velliou, & Taoukis, 2011; Wanihsuksombat et al., 2010). The temperature-dependent performance of a TTI system has been investigated previously using the Arrhenius equation (Ellouze & Augustin, 2010). Fick's law of diffusion can also be applied to establish a kinetic model with regards to the TTI diffusion rate (Galagan, Hsu, & Su, 2010).

Angelica keiskei is a well known herbal plant in Asian countries. Green juice extracted from fresh leaves of this plant is used as a functional food drink that is known for nutritional and health benefits (Akihisa et al., 2003; Kim et al., 2014; Zhang, Yamashita, Yasuda, Yamamoto, & Ashida, 2015). Fresh angelica juice reaches consumers without pasteurization mostly through a home delivery service and is recommended for consumption within 48–72 h with storage at 5 °C (Pulmuone, 2015). However, temperature deviations occur from specified values during product transport, handling, and storage, particularly when consumers store the product at home during summer. Such temperature variations put this product at risk of microbial contamination. Previous studies reported that unpasteurized vegetable juices can support growth of microorganisms (Song et al., 2006; Zhou, Wang, Hu, Wu, & Liao, 2009). On the other hand, there are concerns about nutritional and sensory quality deterioration in vegetable juices after thermal pasteurization (Song et al., 2007).

The aim of this study was to establish a model for prediction of the response of a diffusion-based TTI system and to evaluate applicability of the TTI system as an indicator of threshold microbial growth in NPA juice or similar types of perishable food products due to temperature abuse during storage and distribution. A mathematical kinetic model was established based on measurement of the time-temperature dependent diffusion distance of a TTI at different temperatures. Predicted diffusion distances were then compared with measured values to validate the kinetic model. Finally, the relationship between TTI response and microbial growth in NPA juice was investigated under both isothermal and dynamic temperature conditions.

# 2. Materials and methods

#### 2.1. Materials

Non-pasteurized angelica (NPA) juice (pH = 6.12) was obtained directly from Pulmuone Health & Living (Jeungpyeong, Republic of Korea). Isopropyl palmitate (IPP) (model: Hipure 13T) was provided by OhSung Chemical Ind. Co., Incheon, Republic of Korea. Prototype TTIs based on diffusion of IPP were provided by Inditech Korea Co., Hwaseong, Republic of Korea. The IPP type of TTI has a multilayered structure (maximum diffusion distance: 41 mm; width: 5.0 mm; thickness: 0.2 mm) comprised of a red bottom layer and an upper microporous film (3M, St. Paul, MN, USA) (Fig. 1). The microporous film is originally opaque white (non-transparent). As IPP diffuses and fills micropores in the film, light passes through oil-containing pores and the IPP containing film becomes transparent, revealing the color of the bottom layer. The diffusion of IPP from the injection site can be clearly observed as IPP melts and diffuses from the injection site as a function of time and temperature (Park, 2010).

### 2.2. Experimental design and storage conditions

TTI prototypes were stored at isothermal temperatures of 15, 20, 25, 30, and 35 °C for 48 h to establish a kinetic response model. The temperature was recorded using a data logger (QuadTemp 2000, MadgeTech, Inc., Contoocook, NH, USA). Validation of the kinetic model was accomplished using TTI storage at three isothermal temperatures of 13, 23, and 33 °C. NPA juice samples taken directly from the manufacturing plant were used to enumerate initial microbial counts. Other juice samples were stored isothermally at 5, 15, and 25 °C (278, 288, and 298 K), respectively, with the TTI to investigate correlations between microbial growth in NPA juice and TTI response. Temperature fluctuations between 5 °C (refrigeration temperature) and 25 °C (room temperature) were used for simulation of dynamic storage conditions. NPA juice storage at 5 °C for 6 h, followed by storage at 25 °C for 6 h, was repeated 4 times. Temperature was recorded using a temperature logger (TL20, 3M, St. Paul, MN, USA) that remained with juice samples during the dynamic storage period.

# 2.3. Characterization of the isopropyl palmitate (IPP) diffusionbased TTI

# 2.3.1. Differential scanning calorimetry (DSC)

A DSC 8000 differential scanning calorimeter (Perkin Elmer, Waltham, MA, USA) was used for the measurement of the melting point of IPP. Samples of IPP and an empty stainless steel pan as a reference were heated from -10 °C to 60 °C at a rate of 5 °C/min. Nitrogen was used as a purge gas at a flow rate of 50 mL/min. Samples were then allowed to cool to -10 °C, followed by reheating to 60 °C at the same rate. The calorimeter was calibrated using indium and zinc as standard reference materials.

# 2.3.2. Measurement of the isopropyl palmitate (IPP) diffusion distance in the TTI

IPP (200  $\mu L)$  was injected into a TTI well made with a cotton pad (10  $\times$  4  $\times$  2 mm) and the hourly diffusion distance was measured using images recorded with a digital single-lens reflex camera (EOS500D, Cannon, Tokyo, Japan). The camera and the TTI were set



Fig. 1. Schematic diagram and photos showing isopropyl palmitate (IPP) diffusion through microporous film in a TTI system (a) before and (b) after injection of IPP.

in a low-temperature incubator (SC-LIB150, Sungchan Science Co., Pocheon, Republic of Korea) to establish isothermal and dynamic temperature conditions. Eight TTI devices were used for each storage temperature and experiments were performed in triplicate.

#### 2.4. Microbial analysis

The microbiological quality of NPA juice was evaluated based on measurement of total aerobes every 6 h for 48 h under both isothermal and dynamic storage temperatures. Juice microbial enumeration involved ten-fold serial dilution in a sterile 0.85% NaCl solution. Total aerobes were enumerated using the pour plate method on an aerobic count plate (Petrifilm™, 3M, St. Paul, MN, USA) and colonies were counted using a plate reader (3M, St. Paul, MN, USA) after 48 h of incubation at 35 °C. Microbial analyses were carried out in triplicate using three samples for each storage temperature.

#### 2.5. Modeling approach

# 2.5.1. Kinetic model of time-temperature dependent diffusion in the TTI

In order to establish a TTI diffusion model, several equations relative to diffusion time and temperature were used. A major advancement in diffusion theory was based on the work of Adolf Eugen Fick who developed the first law of diffusion and defined a total one-dimensional flux J as Eq. (1) where D is the diffusion coefficient, C is the concentration of the diffusing substance, x is the coordinate perpendicular to the section, and J is the flux per unit area (Table 1) (de Beer, Stoodley, Roe, & Lewandowski, 1994).

Another equation for diffusion, derived from Eq. (1) and known as Fick's second law, is used to determine the amount of a diffusing substance in a medium when diffusion is one-dimensional. The equation is represented as Eq. (2) where  $C \pmod{m^3}$  is the concentration of the substance, t (s) is time,  $D \binom{m^2/s}{s}$  is the diffusion coefficient, and  $x \pmod{m}$  is the distance (Table 1) (Geankoplis, 1995). Fick's second law was applied to the TTI system on the assumption that surface resistance was negligible for non-steady-state diffusion.

According to the average value of the squared distance  $X^2$  (Galagan et al., 2010), the diffusion coefficient does not depend on the substance concentration. Hence, the  $X^2$  value of diffusion with time (*t*) was determined using Eq. (3) (Table 1). The distance and time for diffusion were known, so the coefficient of diffusion was determined as Eq. (4) (Table 1).

The IPP diffusion rate was studied at 288, 293, 298, 303, and 308 K and a kinetic model equation for IPP diffusion was determined. In order to confirm the temperature dependence of diffusion, the IPP diffusivity (*D*) value and the absolute temperature (*T*) were plotted using a modified Arrhenius equation, as Eq. (5) where *D* is the diffusion coefficient,  $D_o$  is a frequency factor, *R* (8.314 J mol<sup>-1</sup> K<sup>-1</sup>) is the universal gas constant,  $E_a$  (kJ/mol) is the

activation energy, and T (K) is absolute temperature (Table 1) (Li, Liu, Liang, Li, & Zhang, 2008). The curve fitting and regression analysis were performed using IBM SPSS Statistics 21.

#### 2.5.2. Model of microbial growth

A model reflecting microbial growth in juice was required for precise evaluation of the microbiological quality of juice in relation to TTI diffusion because microbial counts can fluctuate due to experimental error. Exponential growth of total aerobes was expressed in linear form in log count versus time plots. Therefore, the population of total aerobes was plotted and linear regression was performed using exponential growth phase data, as Eq. (6) where N (CFU/mL) is the juice microbial count, N<sub>0</sub> (CFU/mL) is the initial microbial load, *a* is the slope, *b* is an intercept, and *t* (h) is time (Table 1) (Pérez-Rodríguez & Valero, 2013).

#### 3. Results and discussion

#### 3.1. Diffusion in the TTI under isothermal conditions

Monitoring and recording of temperature conditions during distribution and storage of perishable food products is important. Variations in temperature can affect food safety and quality. A TTI provides a visual summary of the temperature history of a product, translating the overall effect of time and temperature into a visually simple presentation (Pavelková, 2013). A diffusion-based TTI uses temperature-dependent diffusion of a colored chemical substance, such as a fatty acid ester, through a porous matrix made of highquality blotting paper. The measurable response is the distance of the proceeding diffusion front of fatty acid from the origin (Kerry, O'Grady, & Hogan, 2006). The diffusion of the fatty acid ester may depend on different factors, such as time and temperature, resulting in a clear and instantaneous color change. In addition, the maximum diffusion end-point can be established using experimentation and mathematical modeling for prediction of the shelflife of a specific food product (Vaikousi, Biliaderis, & Koutsoumanis, 2009).

Differential scanning calorimetry (DSC) is a thermoanalytical technique used for direct measurement of the heat capacity of polymeric materials, which takes place within a regulated increase or decrease in temperature. DSC provides the melting temperature of a material from a calibrated and highly precise system. DSC has wide applications in industry for evaluation of sample purity and stability. The presence of an impurity in a material can affect the melting point. Moreover, DSC not only indicates the onset of melting, but also the peak temperature that corresponds to complete melting in organics and the energy that the melting transition requires in order to occur. The results of a DSC experiment comprise a heating and a cooling curve (Gill, Moghadam, & Ranjbar, 2010; Gregorova, 2013). DSC thermograms revealed characteristic transitions (first-order melting, and second-order endothermic transition) occurring in IPP (Fig. 2). The peak corresponding to transition

Table 1

The equations used for fitting the IPP diffusion activity and microbial growth of NPA juice.

No.	Name of the model	Equation	Reference
1	Fick's first law of diffusion	$J = -D \frac{dC}{dx}$	de Beer et al. (1994)
2	Fick's second law of diffusion	$\frac{\partial C}{\partial t} = -D \frac{\partial^2 C}{\partial x^2}$	Geankoplis (1995)
3		$X^2 = 2Dt$	
4		$D = \frac{X^2}{2t}$	
5	Modified Arrhenius equation	$\ln D = -\left(rac{E_a}{R} ight)rac{1}{T} + \ln D_0$	Li et al. (2008)
6		$\log \frac{N}{N_0} = at + \log b$	Pérez-Rodríguez and Valero (2013)
7		$\ln D = -3760.3 \frac{1}{T} - 8.5906$	
8		$X = \sqrt{2t} \times e^{-3760.31 - 8.5906}$	



Fig. 2. DSC thermogram showing the melting point of isopropyl palmitate (IPP) and description of temperature abuse for NPA juice, and the corresponding TTI system response.

at a temperature of 13.5 °C (onset of melting) showed the melting point of IPP (14.8 °C) used in this study. The DSC thermogram melting peak for IPP reported herein was similar to results reported by Klar and Urbanetz (2009). Furthermore, no TTI activity was observed below a temperature of 13.5 °C in this study, in agreement with DSC experiment results.

The diffusion rate of IPP was studied under five different isothermal conditions (Fig. 3). After 48 h of storage, the final diffusion distances were 11.1, 12.8, 14.5, 16.0, and 17.5 mm at 15, 20, 25, 30, and 35 °C, respectively. The diffusion rate was faster in the beginning, but velocity declined with time. Faster diffusion occurred at higher temperatures, indicating that TTI diffusion was both time and temperature dependent and that IPP was suitable for use in diffusion-based TTI systems. However, the proposed TTI was only verified for indication of temperature abuse above 13.5 °C (Fig. 2). Diffusion of IPP may also depend on the temperature sensitivity of IPP, the polymer concentration, and the film matrix. However, these factors can be controlled and are mostly nonvariable. IPP is also suitable for reduction of capillary forces in the film matrix (Klar & Urbanetz, 2009). Polymer-based TTI systems can provide a good indication of temperature and time fluctuations during different stages of food processing and storage (Lee & Shin, 2012).

TTI devices are attached directly to a food package surface. Therefore, the chemical safety of IPP must be addressed. The possibility of skin irritation caused by a variety of external stimuli has been raised (Kawahara & Tojo, 2007). IPP, on the other hand, has been used as an emollient in cosmetics due to adsorption characteristics (Bhatia, Ahmad, Mohamed, & Chin, 2006). Furthermore, the TTI device used in this study was designed in such a way that IPP is never in direct contact with the consumer or the package. Similarly, diffusion-based TTI systems have been developed and studied previously (Arens et al., 1997; Prusik, Arnold, & Fields, 2000). Different diffusion-based TTI systems have used a visible dye dissolved in a migrating fluid. However, the TTI system used in this study was based on a transparent IPP that renders microporous film transparent after absorption, revealing the underlying color due to IPP diffusion.

#### 3.2. Kinetic model prediction for the diffusion-based TTI system

TTI diffusion coefficients (*D*) for IPP at 288, 293, 298, 303, and 308 K (15, 20, 25, 30, and 35 °C, respectively) were calculated using Eq. (4). Diffusion distances for different temperatures (*D* values) are shown in Table 2. Plots of  $\ln D$  versus 1/T were prepared and a linear regression was performed (data not shown). Table 3 shows analysis



Fig. 3. The diffusion distance of isopropyl palmitate (IPP) through a TTI film during 48 h of storage at 5 different isothermal temperatures.

#### Table 2

Diffusion coefficients for isopropyl palmitate (IPP) injected into a TTI system at different temperatures.

Temperature		Diffusion coefficient (m <sup>2</sup> /s)
К	°C	
288	15	$3.860  imes 10^{-10}$
293	20	$5.026  imes 10^{-10}$
298	25	$6.346  imes 10^{-10}$
303	30	$7.716  imes 10^{-10}$
308	35	$8.966  imes 10^{-10}$

of variance (ANOVA) of the regression. The regression equation used was y = -3760.3x - 8.5906 with an  $R^2$  value of 0.9925. Moreover, ANOVA results showed F-values of 394.80 and P-values of 0.0003. Thus, the TTI system was correlated with time and temperature. As a result of linear regression, Eq. (5) can be rewritten as Eq. (7) (Table 1).

Hence, the equation for prediction of the IPP diffusion distance in a TTI system can be established by re-arranging Eq. (3) as Eq. (8) where *X* represents the diffusion distance (m), *t* is time (s), and *T* is temperature (K) (Table 1). Eq. (8) indicates the relationship between diffusion distance and time-temperature. This model can be used for prediction of the diffusion distance in TTI systems based on adjustment of specific time and temperature values. Mathematical models involving time and temperature as major predictive variables in a TTI system have been established in other studies using enzymes (Wu et al., 2015), polymers (Lee & Shin, 2012), proteins (Grauwet, Plancken, Vervoort, Hendrickx, & Loey, 2010), and biological-based TTI systems (Ellouze & Augustin, 2010).

# 3.3. Validation of the TTI kinetic model

Evaluation and comparison of a mathematical model using experimental data are essential to obtain an optimal response (Ghafoor, Park, & Choi, 2010). Evaluation of the predictive model of IPP diffusion in a TTI system was accomplished based on comparison of the diffusion distance predicted using Eq. (8) with experimentally measured distances at temperatures of 13, 23, and 33 °C. Predicted curves coincided well with experimental curves, resulting in high concordance rates of 95.3, 97.8, and 96.7% at 13, 23, and 33 °C, respectively (Fig. 4). The model established in this study effectively represented IPP behavior in the diffusion-based TTI system.

# 3.4. Microbial growth in NPA juice and the TTI response under isothermal conditions

The microbial quality of juice was investigated to demonstrate the effectiveness of diffusion-based TTI for indicating the microbial quality of juice. The TTI diffusion distance of IPP and growth of aerobic bacteria in juice under isothermal storage at 5, 15, and 25 °C for 48 h are shown in Fig. 5. Counts for total aerobes did not increase at 5 °C for 48 h (Fig. 5a), remaining static at the initial level of 3.7 log CFU/mL (Fig. 6). This high initial microbial count occurred because thermal processing of juice is not used in order to maintain high nutritional benefits (Song et al., 2007). However, the initial microbial load of a juice can vary depending on different factors. The onset of melting of IPP was 13.5 °C (Fig. 2). Therefore, no TTI response (no IPP diffusion) was observed at 5 °C for 48 h. The total aerobic count of NPA juice samples also remained sufficiently below the critical level under this isothermal condition.

A storage temperature of 5 °C that is required for vegetable juices is lower than the usual cold chain temperature (Song et al., 2006). NPA juice manufacturers recommend storage of juice products at 5 °C, but usual cold chain temperatures are generally higher. Furthermore, minor temperature fluctuations can occur in a stored product (James, 2006) and normal deviations of  $\pm 0.5$  °C are expected from the set point for food distribution (EN12830, 1999). Angelica juice and other foods requiring a storage temperature of 5 °C can be exposed to higher distribution temperatures and, thus, IPP is suitable for TTI systems intended for products exposed to elevated refrigeration temperatures. The handling temperature of a refrigerated product can reach as high as 13.5 °C in some cases, indicating non-standard storage or distribution, which may not be acceptable. Melting and freezing of IPP started at approximately 13.5 °C. Hence, IPP can respond to temperature abuse of refrigerated products at temperature above 13.5 °C (Fig. 2).

Total aerobic counts in NPA juice started to increase after 12 h of storage at 15 °C (Fig. 5b). An aerobic count of 6 log CFU/mL was regarded as critical, and fresh juices with microbial loads less than 6 log CFU/mL can be acceptable for microbiological safety (Feng, Ghafoor, Seo, Yang, & Park, 2013). Bacterial counts during the logarithmic growth phase (12–48 h) were subjected to linear regression analysis to determine the storage time when juice microbial levels become critical. The time for angelica juice to attain the threshold microbial load at 15 °C was 36.6 h when the total aerobic count became 6 log CFU/mL, or 2.3 log (N/N<sub>0</sub>) CFU/mL (Fig. 5b). The TTI diffusion distance covered by IPP was 9.7 mm after 36.6 h.

NPA juice stored at 25 °C showed a rapid increase in total aerobic counts after 6 h, and bacteria reached the stationary phase after 24 h (Fig. 5c). Based on linear regression developed using data obtained during the logarithmic growth phase (6-24 h) at 25 °C, total aerobe counts in juice were predicted to be 6 log CFU/mL at 12.5 h with a predicted diffusion distance of 7.2 mm. These results indicated an appearance of color and a diffusion distance of 7.2 mm on the microporous film at 25 °C, signifying spoilage by microorganisms. However, the initial microbial load of juice may vary due to different factors that include handling, sanitation, and the microbial load of fruits used in juice preparation. Hence, the initial microbial load of food packaged in a TTI-system should be established for prediction of the time until the critical microbial load is reached on the basis of microbial growth in response to storage temperature and an association with IPP diffusion in the TTI system. Hence, results of this study cannot be generalized for different products and variable handling and storage conditions. However, results presented herein can serve as a basis for further studies for individual products and different cases in the food chain.

Ta	bl	e	3	

ANOVA for the linea	r regression	analysis o	f diffusivity	against	temperature.
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Source	Regression coefficients	Standard error	Sum of squares	df	Mean square	F-values	P-values
Regression			0.449	1	0.449	394.801	0.0003
Constant	-8.591	0.636					0.0009
(1/T)	-3760.3	189.249					0.0003
Residual			0.003	3	0.001		
Total			0.453	4			



**Fig. 4.** Comparison between measured diffusion distances ( $\bullet$ ) and predicted diffusion distances (-) at different isothermal storage temperatures.

# 3.5. Microbial growth in NPA juice and the TTI response under dynamic conditions

Storage temperature, one of the major factors influencing the rate of microbial development in foods, often deviates from specifications. This limitation is generally recognized by industrialists. retailers, and even food authorities (Ellouze & Augustin, 2010). In this study, the prototype TTI system was evaluated for effectiveness in identifying the microbial quality of juice not only under isothermal conditions, but also under dynamic temperature conditions. Dynamic conditions were simulated based on alteration of the storage temperature between 5 and 25 °C for simulation of refrigeration and room temperatures, respectively. Four cycles of 6 h storage at 5 °C and 6 h at 25 °C were applied to juice samples for 48 h. The NPA juice microbial growth pattern and IPP diffusion distance during this dynamic storage period are shown in Fig. 6. The total aerobe count increased gradually and reached 9.1 log CFU/ mL at the end of the storage period. Microbial growth showed a noticeable increase when the storage temperature was 25 °C, but did not show a significant increase at 5 °C as refrigerated storage can reduce microbial growth rates in foods.

Lu et al. (2013) reported development and application of an enzyme reaction-diffusion TTI system for prediction of the shelf-life of perishable foods during cold chain transportation. Chun, Choi, Lee, and Hong (2013) reported use of an enzymatic TTI system for evaluation of quality parameters of beef patties subjected to different storage temperatures (5, 15, and 25 °C) in which an insufficient correlation with change in TTI color was observed at storage temperatures of 5 °C and 15 °C. Design of individual TTI systems based on storage temperature, time, and type of meat was emphasized.

Due to a high melting point, IPP did not show any diffusion at 5 °C; however, IPP diffused easily at 25 °C. Microbial growth and IPP diffusion showed similar increases after 12 h, and the diffusion distance before the critical microbial growth time (27.4 h) was nearly 7.6 mm, in close agreement with the diffusion distance under isothermal conditions. The total aerobic count at this time was 6 log CFU/mL. Based upon observed microbial growth in relation to TTI response under isothermal and dynamic conditions, IPP diffusion of 7.0 mm in the TTI system can be used as a threshold point for microbial spoilage of NPA juice. A diffusion-based TTI system using IPP can be used for prediction of the microbiological quality of NPA juice during storage under non-isothermal and isothermal conditions provided that careful monitoring of storage time and



**Fig. 5.** Comparison of diffusion of isopropyl palmitate in a TTI system with growth of total aerobic bacteria in non-pasteurized angelica juice during isothermal storage at 5 °C (a), 15 °C (b), and 25 °C (c) for 48 h.

temperature is performed for a precise prediction of the microbial quality of juice based on the TTI response. Refrigerated storage of foods prevents exponential growth of microorganisms over a short period. The probability of microbial hazard increases when refrigerated foods are left at higher than recommended refrigeration or room temperatures and, in such cases, the IPP-based TTI system responded well. The TTI system characterized herein needs to be improved with consideration of different aspects of food processing, storage, handling, and transportation.

In recent years, conventional food packaging systems have become unsuitable for use in the modern food industry. Hence, active and intelligent food packaging systems are in demand by modern consumers for high quality and nutritious foods. Active and



Fig. 6. Correlation between diffusion distance in a TTI system and total aerobic growth in non-pasteurized angelica juice under dynamic temperature conditions; 4 cycles of 6 h each at 5 °C and 25 °C.

intelligent food packaging systems are more dynamic and functional than conventional counterparts (Lee, Lee, Choi, & Hur, 2015). Kang et al. (2014) reported the use of TTI systems as cheap and userfriendly alternatives to existing practices for control of quality variations in kimchi due to non-uniform temperature distribution during storage and transportation. TTI systems are efficient and economical tools for monitoring, recording, and translating the overall effect of temperature history on food safety and quality at a product unit level (Chun et al., 2013; Ellouze & Augustin, 2010; Wu et al., 2015). The TTI system used in this study is cost-effective with good commercialization potential.

# 4. Conclusions

A model was established for prediction of the response of a diffusion-based TTI system, and for evaluation of applicability of the TTI system as an indicator of threshold microbial growth in NPA juice due to handling under non-standard temperatures. The kinetic model developed in this study showed a high predictive reliability under isothermal storage conditions. Results predicted using the model coincided with experimental results. Total aerobic counts revealed that the time and temperature dependence of bacterial growth in NPA juice, and the TTI response, were correlated under both isothermal and dynamic storage conditions. IPP diffusion of more than 7.0 mm in the TTI system presented in this study can be a threshold point for microbial spoilage of NPA juice. However, the proposed TTI system was only verified for indication of temperature abuse above 13.5 °C. Diffusion-based TTI systems can serve for monitoring microbial quality and temperature abuse during storage and transport of individual packages of perishable food products. The diffusion-based TTI system reported herein showed good reliability for prediction of the microbial quality of perishable food products during distribution and storage.

#### **Conflict of interest**

The authors declare no conflict of interest.

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