Kinetic Modeling Of A Bi-Enzyme Time-Temperature Indicator (TTI) Based on Different Parameters for Monitoring Food Product Quality

Qiang Li*, Min Duan#, Wen Liu#, Yue Dai#, Ren Li$	ext{d}$

Institute of Food and Agriculture Standardization, China National Institute of Standardization, Beijing, China

*Corresponding author e-mail: liqiang@cnis.gov.cn, duanmin@cnis.gov.cn, bliuwen@cnis.gov.cn, daiyue@cnis.gov.cn, liren@cnis.gov.cn

Abstract. Time-temperature indicator has been widely applied in the food industry. And, the theoretical study shows that if the time-temperature indicator is applied to monitor food, the activation energy of TTI temperature-dependent must be consistent with the activation energy of food quality loss. In this study, kinetics of absorbance and color changes in bi-enzyme were investigated as a function of storage temperature. Absorbance value was used to evaluate the enzyme reaction process under different temperature. Color change was evaluated in terms of the total color difference (DE). The results indicated that the time - temperature dependence of Absorbance and DE values followed a zero-order model. The Arrhenius equation adequately described the temperature dependence of the reaction rate constants for both absorbance and color parameters, respectively, from which the activation energies and rate constant at different temperature were obtained. There were significant linear correlations between absorbance, color and time parameter.

1. Introduction
After the food production is completed, its quality is mainly affected by the length of transportation storage and the temperature of external environment [1].

A time - temperature indicator (TTI) is affixed to the food package and undergoes the same time-temperature accumulation as the food. TTI indicates the dual effects of time and temperature experienced by the food in the circulation process through a simple mechanical deformation or color change, so that the TTI can monitor the food quality change and the remaining shelf life [2,3]. TTI can be used in perishable chilled foods to monitor the all or part temperature history of the food from the completion of production to the sale to ensure food quality and safety while avoiding economic losses and waste.

The development of TTI in foreign countries is far earlier than that in our country, and it is going to be commercialized step by step, for example, TTI was applied to frozen fish [4], frozen pork [5], milk [6], frozen burger [7], frozen fruits and vegetables [8]. According to the working principle, TTI is divided into enzyme type, microorganism type, polymer type, electron type, diffusion type and so on [9], in which the enzyme type TTI is simple and easy to control [3]. Kim and Park [10,11] studied the...
TTI and its kinetic characteristics-based laccase as the reaction system. Microorganism TTI was prepared by Dong [12], Park [13] and Kim [14] using screen printing technology for monitoring the shelf life of fresh pork and chicken. Zabala made a time-temperature-type smart label using rotary printing technology and also studied its kinetic characteristics [15].

At present, there are more researches on enzyme indicator in China. Enzyme indicator produces obvious visible color change with the accumulation of time and temperature through the reaction of enzyme and substrates, so as to indicate the time and temperature process of food [16]; Wu [17] used urease as the reaction system of TTI. Tian studied an enzymatic TTI based on protease reaction [18]; Lu studied the basic lipase type TTI, through pH change of the reaction system combined with acid and alkali indicator to made a time temperature indicator [19,20,21]. Ge preliminarily studied the tyrosinase-based time-temperature indicator system [22]. Sun [23] developed the amylase type TTI. At the same time, Qian [24] and Feng [25] developed glucoamylase TTI for the monitoring of chilled meat and immobilized enzyme TTI for the monitoring of yogurt.

In this study, a time-temperature indicator was developed using glucose oxidase and horseradish peroxidase as reaction systems. The principle of the time-temperature indicator was based on Arrhenius kinetic equation. In a certain temperature range, the response value and the change of food quality follow Arrhenius criterion at the same time. Taooukis and labuza [26] proposed in 1989 that TTI can be applied to a food product only if the difference between TTI and Activation energy (Ea) value of the food product is less than 40 kJ / mol, and the estimation error of the food product quality is controlled below 15%. Later, taoukis proposed in 2001, when the absolute value between the Ea value of food and the Ea of TTI is less than 25 kJ / mol, TTI can be more accurately applied to the food. That is, when the Ea value between the chemical reaction system of TTI and the food quality change system is the same, the response function and the food quality change function have a certain linear relationship, and the indication value can reflect the food quality change. The developed indicator system can simulate and indicate the change of food quality based on different response values, the response function and kinetic parameters of different response values (absorbance and color change values) were determined. The activation energy was not different, which further proved that the indicator can be used for a class of cold fresh food accurately.

2. Materials and methods

2.1. Materials
Glucose, Tetramethylbenzidine Sigma Co., Ltd.; Glucose Beijing Kehua Jingwei Technology Co., Ltd.; Glucose oxidase (10KU) Beijing Kehua Jingwei Technology Co., Ltd. Horseradish catalase (10KU) AMRESCO (U.S.), TMB AMRESCO (U.S.), Anhydrous ethanol Beijing Chemical Plant, Dihydrogen Phosphate Dihydrogen Phosphate Chemical Group Beijing Chemical Co., Ltd., Sinopharm Chemical Reagent Beijing Co., Ltd., Disodium hydrogen phosphate dodecahydrate Sinopharm Chemical Reagent Beijing Co., Ltd.,

2.2. Preparation of bi-enzyme based TTI
The concentration of Glu, TMB, god and HRP were 0.35 mg / ml, 0.15 mg / ml, 4 μg / ml and 0.5 μg / ml, respectively, which were added into the beaker. the solution was shaken uniformly and reacted at different temperatures (0.20 40 °C). The absorbance at 450 nm and the color change value (DE) were measured by color difference instrument. Three groups of parallel experiments were conducted for each group.

2.3. Enzyme reaction kinetic analysis
According to theoretical studies, two conditions are needed to meet for applying the time-temperature indicator onto food: the end of the TTI reaction must be consistent with the end of the shelf life of food; the characterized TTI to temperature sensitivity activation energy must be equivalent to the activation energy lost from food quality.
In 1989, Taoukis and Labuza proposed that TTI should be applied to the food only if the estimated error of the food quality was below 15% when the difference between the $E_a$ values of TTI and food was less than 40 kJ/mol. Later, Taoukis proposed again in 2001 that the TTI could be applied to the food more accurately when the difference between the activation energy $E_a$ of the food and the activation energy $E_a$ of the TTI was less than 25 kJ/mol.

2.4. Reaction kinetics based on reaction rate

The substrate solution and enzyme solution in the aforesaid ratios were added sequentially into 5 clean 10 ml centrifuge tubes according to the optimized best system parameters, then the 5 tubes were placed into water bath at 0, 4, 20 and 40°C respectively, the absorbance was measured every 1 min for the first 45 min (with deionized water as the standard sample). Arrhenius equation was used to analyze its reaction kinetics.

2.5. Reaction kinetics based on color difference

CIELab color system is used for the study of dynamic color change in TTI and use corresponding value $F(X)$ ($F(X) = D_b$) as the dynamic parameter. The $D_b$ value is the color difference from blue to yellow between the start and each interval.

TTIs were incubated at 0, 20 and 40°C respectively. The TTI color variation at different temperatures with time corresponds to $F(X)$ is determined by ColorQuest XE (HunterLab, USA).

According to the indicator kinetics described by Taoukis and Labuza, the color response of the time temperature indicator $F(X)$ can be expressed as follows:

$$F(X) = k dt$$  \hspace{1cm} (1)

K is the reaction rate constant and t is the reaction storage period (h).

By plotting the curve of the response value $F(X)$ versus time, a straight line is obtained and the value of k at different storage temperatures can be calculated from the slope.

The Arrhenius equation can be used to calculate the temperature dependence of the reaction (Equation (2)). Using equation (2), we get the natural logarithm of the Arrhenius function:

$$\ln k = \ln k_0 - \frac{E_a}{RT}$$  \hspace{1cm} (2)

Get a straight line by plotting $\ln k$ against $1 / T$. The activation energy $E_a$ can be calculated from the slope and the pre-exponential factor $k_0$ from the intercept.

Adding the substrate solution and the enzyme solution in the aforesaid ratio into 3 clean beakers after expanding the reaction volume 20 times, then three beakers were placed in ice bath, room temperature and 40°C water bath respectively for reaction, color difference was measured every 2 min for the first 30 min (with deionized water as standard sample), and every 5 min for 30-80 min, every 10 min for 80-100 min, Arrhenius equation was used to analyze its reaction kinetics.

3. Results and discussion

3.1. Reaction kinetics analysis based on absorbance

According to the kinetic description of TTI by Taoukis and Labuza, the relationship between the $F(X)$ of the absorbance $X$ of the TTI system and the apparent reaction rate (i.e., the rate of increase of the absorbance of the product) $k$ can be expressed by the following equation:

$$F(X) = kt$$  \hspace{1cm} (3)

Where, $F(X)$ - responsive function of TTI; t - time; k - apparent reaction rate constant.
In this experiment, the curve of absorbance value varying with time of TTI during the indicated time could be plotted by measuring the absorbance value of the time-temperature indicator in the process of color change reaction. The absorbance-time curve was fitted by Origin software, and the reaction rates of glucoamylase time-temperature indicator at different temperatures can be obtained.

Figure 1. The relationship between the absorbance of TTI and the time at 0°C, 4°C C, 20°C C, 30°C and 40°C
As shown on Fig. 1, the reaction speed increased with increasing temperature, and the absorbance value and time showed a significant linear relationship:

\[ y = a + b \cdot x = a + b(kt) \]  \hspace{1cm} (4)

Transform Equation 4 to get the expression of Formula 5:

\[ F(X) = kt = \frac{(y-a)}{b} \]  \hspace{1cm} (5)

Therefore, in the bi-enzyme TTI system, \( F(x) \), the response function of TTI, was \( \frac{(y-a)}{b} \).

From the fitted curve parameters in Fig. 1, the reaction rate of the TTI system and the correlation coefficient of the fitted curve at this temperature could be obtained as shown in the table.

| Table 1. The reaction rate \( k \) of the TTI system and the correlation coefficient of the fitting curve at different temperatures |
|---|---|---|---|
| T/K | \( k/\text{min}^{-1} \) | \( R^2 \) |
| 273 (0°C) | 0.021 | 0.99142 |
| 277 (4°C) | 0.037 | 0.98937 |
| 293 (20°C) | 0.050 | 0.99685 |
| 303 (30°C) | 0.095 | 0.99708 |
| 313 (40°C) | 0.109 | 0.98237 |

It could be seen from Table 1 that the correlation coefficients \( R^2 \) of the fitted curve equations under different temperatures are all above 0.98, indicating that the exponential correlation of absorbance value of the TTI at different temperatures and time was significant.

In order to visualize the reaction rate of the TTI system at different temperatures, the response curve \( \frac{(y-a)}{b} \) versus time it was plotted and as shown in Figure 2 below. It could be seen from the figure that the higher the temperature, the steeper the curve of \( \frac{(y-a)}{b} \) to \( t \), indicating that the larger the reaction rate \( k \) was.

![Figure 2. TTI response function on the time curve at 0, 4, 20, 30, 40°C](image)
The effect of temperature on the reaction rate in the enzymatic catalytic reaction follows the Arrhenius formula.

\[
\ln k = \ln k_0 - \frac{E_a}{R} \frac{1}{T} \tag{6}
\]

In the formula, \( k \) is the reaction rate constant, \( k_0 \) is the pre-exponential factor. \( R \) is the molar gas constant, \( R = 8.314 \text{ J/(mol} \cdot \text{K}) \)

\( T \) is the thermodynamic temperature; \( E_a \) is the activation energy.

Table 2. Temperature and \( \ln k \) corresponding value of TTI

| T/K  | \( \ln k \)  | 1/T      |
|------|--------------|----------|
| 273 (0°C) | -3.86323     | 0.003663 |
| 293 (20°C) | -2.99573     | 0.003413 |
| 313 (40°C) | -2.21641     | 0.003195 |

According to the \( \ln k \) value corresponding to the different temperatures in the above table, a relation curve between \( \ln k \) and 1/T was made as shown in the following figure 3.

![Figure 3. \( \ln k \) and 1/T relationship curve of TTI](image)

The linear correlation coefficient of \( R^2 \) of the model is 0.99985, indicating the linear relationship between TTI \( \ln k \) and 1/T. The straight line fitted by Origin is

\[
\ln k = 9.01817 - 3517.66 \frac{1}{T}
\]

According to the Arrhenius equation, \( R = 8.314 \), from this we can get that the activation energy is

\( E_a = 29.245 \text{ kJ/mol} \)

Theoretical studies show that the TTI can be applied to the product when the difference of the activation energy of the TTI is within 25 kJ/mol from that of the product. Therefore, the indicator can indicate a product with the activation energy in the range of 4 kJ/mol to 54 kJ/mol.
3.2. Enzymatic Reaction Kinetic Result Analysis Based on Chromatism of System

Figure 4A. The color change of Enzyme system in TTI

Figure 4B. The relationship between the color of the system over time at different temperatures

The change in TTI color over time is shown in Fig. 4A. And according to Fig.4B, we could see that there was a good linear relationship between the color of the system and the time by studying the relationship between the color change of the system and time. Therefore, we could fit the curve of the relationship and got the following results:

Figure 5A. System color and time linear relationship at 0°C
Figure 5B. System color and time linear relationship at 20°C

Figure 5C. System color and time linear relationship at 40°C

Following data can be obtained from the figure:

Table 3. Linear correlation coefficient for ΔE-t at different temperatures

| TTI | \( R^2 \)       |
|-----|-----------------|
|     | \( 0°C \) | \( 20°C \) | \( 40°C \) |
| 1#  | 0.9759          | 0.9946     | 0.9824     |

Table 4. ΔE-t curve k value and reaction activation energy at different temperatures

| TTI | k       | \( E_A \) |
|-----|---------|-----------|
|     | \( 0°C \) | \( 20°C \) | \( 40°C \) |
| 1#  | 49.05   | 70.92     | 191.6      | 23.94   |
According to the data in Table 4 and the Arrhenius equation 
\[
\ln k = \ln k_A - \frac{E_a}{R} \frac{1}{T}
\]
we plotted the image of \(\ln k - \frac{1}{T}\) (Fig.6), and calculated the slope using with the image, and further worked out the activation energy of the enzymatic reaction.

![Arrhenius plot of In k vs 1/T for TTI](image)

**Figure 6. Arrhenius plot of In k vs 1/T for TTI**

The results showed that there was a good linear relationship between the color change and time, which could be used to study the kinetics of enzymatic reaction. The calculated activation energy of the bi-enzyme system was 23.94 kJ / mol. Therefore, the TTI model can be used to indicate accurately the food quality changes when the activation energy is in the range of 0-48.94 kJ / mol.

### 4. Conclusion

In conclusion, the relationship between TTI color and time was studied on the basis of TTI which has been developed by our team. The dynamic parameters of TTI are determined by experiments and the mathematical model of TTI is described in this work. And the ranges of activation energy from to 0-54 kJ/ mol, and the activation energy calculated from the experiment will be suitable for fresh products of Ea according to 0-54 kJ/ mol, such as some fruits and vegetables, some fish and shellfish, loss in quality due to lipid oxidation, enzymatic reaction, etc.

### Acknowledgements

The authors gratefully acknowledge the Central Fundamental Research and the science and technology project of General Administration of Quality Supervision, Inspection and Quarantine of the PRC (562015z-3996, 2015QK240) for their financial support.

### References

[1] Cai HW, Ren FZ, Zhang HT. Liu W. Research of amylese time-temperature indicator. J Food Sci. 11: 60-63 (2006).

[2] Taoukis PS, Labuza TP. Applicability of Time-Temperature Indicators as Shelf Life Monitors of Food Products. J Food Sci. 54(4): 783-788 (2010).

[3] JIA ZQ, LU LX. Research and application on commercial time-temperature indicator. Food & Machinery. 28(01): 250-253 (2012).

[4] Endoza TFM, Welt BA, Otwell S, et al. Kinetic Parameter Estimation of Time-temperature Integrators Intended for Use with Packaged Fresh Seafood. J Food Sci. 69(3): FMS90-FMS96 (2010).
[5] Yoon SH, Lee CH, Kim DY, et al. Time-Temperature Indicator using Phospholipid-Phospholipase System and Application to Storage of Frozen Pork. J Food Sci. 59(3): 490-493 (2010).

[6] Wells JH, Singh RP. Application of Time-Temperature Indicators in Monitoring Changes in Quality Attributes of Perishable and Semiperishable Foods. J Food Sci. 53(1): 148-152 (2010).

[7] Wells JH, Singh RP. Noble AC. A graphical interpretation of time-temperature related quality changes in frozen food. (2006).

[8] Giannakourou MC, Taoukis PS. Systematic; application of time temperature integrators as tools for control of frozen vegetable quality.J Food Sci. 67 (6): 2221-2228 (2006).

[9] Cheng H, Zhu GM, Song R. Research progress of time temperature indicator. Chemical progress. 32 (4): 885-890 (2013).

[10] Park HR, Kim YA, Jung SW, et al. Response of microbial time-temperature indicator to quality in-dices of chicken breast meat during storage. Food Sci Biotechnol. 22(4): 1145-1152 (2014).

[11] Kim E, Choi DY, Kim HC, et al. Calibrations between the variables of microbial TTI response and ground pork qualities.. Meat Sci. 95(2): 362-367 (2013).

[12] Dong YC, Jung SW, Dong SL, et al. Fabrication and Characteristics of Microbial Time Temperature Indicators from Bio-Paste Using Screen Printing Method. Packag Technol Sci. 27(4): 303-312 (2014).

[13] Park HR, Kim K, Lee SJ. Adjustment of arrenius activation energy of laccase-based time – temperature integrator (TTI) using sodium azide. Food Control. 32(2): 615-620 (2013).

[14] Kim K, Kim E, Lee SJ. New enzymatic time–temperature integrator (TTI) that uses laccase. J Food Eng. 113(1): 118-123 (2012).

[15] Zabala S, Castan J, Martinez C. Development of a Time Temperature Indicator (TTI) Label by Rotary Printing Technologies. Food Control. (50): 57-64 (2015).

[16] Reichert H, Simmendinger P, Bolle T. Enzyme-based time temperature indicator: US, US7736866B2 [P]. 2010-07-15.

[17] Wu QM. Development of a Time-Temperature Indicator Reaction System Based on Unease. Hangzhou: Zhejiang University. (2005).

[18] Tian QS. Study on the Application of Variant Enzyme Indicates the Shelf Life of Freshwater Fish[D]. Shanghai: Shanghai Ocean University. (2009)

[19] Ning P, Xu Xl, Fei Y, et al. Study on Time-temperature Integrated Indicator Based on Alkaline Lipase. Acta Agricultural Jiangxi. 20(8): 85-87 (2008).

[20] Lu LX, Cai Y, Zheng WZ. Preparation and Application on Time-temperature Indicator based on Reaction Diffusion of Lipase: China, 201110023063.5 [P]. 2011-09-07.

[21] Wu D. Development of Time-temperature Indicating Chemical reaction system Based on Alkaline Lipase [D]. Hangzhou: Zhejiang University. (2005)

[22] Ge L, Zhu SY, Li ZZ, et al. Preliminary Study on Time-temperature Indicator System Based on Tyrosinase[J]. Science and Technology of Food Industry. (17): 180-184 (2014).

[23] Sun Y, Cai HW, Zheng LM, et al. Development and Characterization of a New AmylaseType Time-temperature Indicator. Food Control. 19(3): 315-319 (2008).

[24] Qian J, Zheng GL, Feng Q. Preparation of Amylase-Based Time Temperature Integrator. Applied Mechanics and Materials. (200): 433-436 (2012).

[25] Feng Q, Qian J, Liu J. Performance of Time-temperature Indicator with Immobilized Amylase. Packaging Engineerin. 35(7): 60-65 (2014).

[26] Labuza, TP, Kamman J. Reaction kinetics and accelerated tests simulation as a function of temperature. Ch.B. In "Applications of Computers in Food Research", (Ed.)I. Saguy, Marcel-Dekker, New York.(1983)