จลพลศาสตร์การเกิดสีน้ำตาลของไข่ขาวระหว่างกระบวนการแปรรูปด้วยความร้อนที่อุณหภูมิสูง

Browning kinetics of egg white during high-temperature process

2

กันต์ฤทัย ต่ายจันทร์[1], สุวลักษณ์ อัศวสันติ[2], ชัยรัตน์ ตั้งดวงดี[1*]
Kanruethai Taichan[1], Suvaluk Asavasanti[2], Chairath Tangduangdee[1*]
รับบทความ: 26 มิถุนายน 2566 แก้ไขบทความ: 25 สิงหาคม 2566 ยอมรับตีพิมพ์: 18 กันยายน 2566

บทคัดย่อ

ผลิตภัณฑ์ไข่ขาวเป็นอาหารที่มีโปรตีนสูง เพื่อให้สามารถเก็บรักษาที่อุณหภูมิห้อง จำเป็นต้องแปรรูปที่อุณหภูมิสูง ส่งผลให้ผลิตภัณฑ์เกิดสีน้ำตาล วัตถุประสงค์ของงานวิจัยนี้ เพื่อพัฒนาแบบจำลองจลพลศาสตร์การเกิดสีน้ำตาลของไข่ขาวที่อุณหภูมิสูงและผลของการเติมเอนไซม์กลูโคสออกซิเดสเพื่อออกซิไซด์น้ำตาลในไข่ขาว ตัวอย่างถูกบรรจุในตลับอลูมิเนียม ให้ความร้อนที่ 70 องศาเซลเซียส เพื่อให้เซตตัวก่อนให้ความร้อนที่ 100, 110 และ 130 องศาเซลเซียส ในอ่างน้ำมัน นาน 5–30 นาที สุ่มตัวอย่างวัดค่าสีด้วยเครื่องสเปคโตรโฟโตมิเตอร์ และคำนวณดัชนีความเหลือง (Yellowness index: YI) ผลการทดลอง พบว่าค่าความสว่าง (L^*) มีค่าลดลง ส่วนค่าสีแดง (a^*) และค่าสีเหลือง (b^*) เพิ่มขึ้นตามอุณหภูมิและเวลาที่ได้รับความร้อน การเปลี่ยนแปลงของค่า YI อธิบายได้ด้วยปฏิกิริยาอันดับที่หนึ่งและสอดคล้องกับผลการทดลอง (R^2=0.92–0.95, $RMSE$=0.11–0.29) นอกจากนี้ ยัง พบว่า การเติมเอนไซม์ 30 units/mL ทำให้ค่าอัตราส่วน YI ลดลงอย่างมีนัยสำคัญ ($p<0.05$) จาก 5.9 เป็น 2.6 ทำให้ได้ผลิตภัณฑ์ไข่ขาวที่มีสีอ่อนลง

คำสำคัญ: ผลิตภัณฑ์ไข่ขาว ปฏิกิริยาสีน้ำตาล เอนไซม์กลูโคสออกซิเดส แบบจำลองจลพลศาสตร์

[1]ภาควิชาวิศวกรรมอาหาร คณะวิศวกรรมศาสตร์ มหาวิทยาลัยเทคโนโลยีพระจอมเกล้าธนบุรี
[2]สมาคมวิทยาศาสตร์และเทคโนโลยีทางอาหารแห่งประเทศไทย (FoSTAT)

Abstract

Egg white product is a high protein product. To produce it as a shelf-stable one, a high temperature is needed, but at the expense of the color attribute being brown. The objective of this research aimed to develop the browning kinetic model of the egg white undergoing a high-temperature treatment and to investigate the effect of adding glucose oxidase to desugar in egg white. The samples were filled in sample holders made of aluminum, then heated at 70 ºC to solidify the egg white. The samples were heated in an oil bath at 100, 110, and 130 °C for 30 minutes. The sample was randomly taken to measure its surface color using a spectrophotometer; the yellowness index (YI) was subsequently calculated. It was found that the L^* value of egg white declined, while a^* and b^* values increased with increasing heating time. The first-order kinetic model was applicable for predicting the change of YI-ratio (R^2 = 0.92–0.95, $RMSE$ = 0.11–0.29). In addition, the samples pre-treated with glucose oxidase of 30 units/mL-egg white and 30 °C-incubation for 6.5 hours had a significant reduction of the YI-ratio ($p<0.05$) from 5.9 to 2.6 leading to obtaining lighter product colorthe quality of CRM. TRM-F-2006 can be used for method validation and quality control purposes.

Keywords: Egg white product, Browning reaction, Glucose oxidase, Kinetic model

[1]Department of Food Engineering, Faculty of Engineering, King Mongkut's University of Technology Thonburi
[2]Food Science and Technology Association of Thailand (FoSTAT)
[*]Corresponding author's e-mail address: chairath65@gmail.com

1. Introduction

Egg white products are often used as ingredients in other food products, usually found in the form of liquid, frozen, and dried egg whites. Nowadays, a change in people's lifestyle influences more consumption of ready-to-eat (RTE) products. Pasteurized hard-boiled egg white contained in a plastic tube or tray has been developed and is considered a high-protein RTE snack. However, it has a short shelf-life and needs to be kept in a refrigerated condition throughout the cold chain resulting in a high operating cost. Sterilization is recognized as an effective method to produce shelf-stable products. A high temperature of sterilization is typically needed for low-acid foods, including egg white; however, using high temperature induces adverse changes in texture, brown color, and undesired flavors, leading to an unacceptable product for the consumer [1].

Melanoidin in the egg white product is an undesired browning compound induced by the Maillard reaction, in which reducing sugar reacts with amino acids when heated at a high temperature [2]. The combination of time and temperature is a significant factor that controls the rate of Maillard reaction. Some previous research studied the color change of egg white during the thermal process but mostly conducted at a temperature below 100 ºC [3–5]. Therefore, the change of egg-white color in a range of sterilizing temperatures is still in doubt. Over the past decade, several studies have attempted to control the Maillard reaction in foods. The general practice is the desugarization of glucose with glucose oxidase (GOX) before heating. The glucose oxidase oxidizes glucose to gluconic acid and hydrogen peroxide, then the hydrogen peroxide is catalyzed by catalase to water and oxygen [6]:

$$2C_6H_{12}O_6 + 2H_2O + 2O_2 \xrightarrow{GOX} 2C_6H_{12}O_7 + 2H_2O_2$$

$$2H_2O_2 \xrightarrow{catalase} 2H_2O + O_2$$

The whiteness and foaming properties of duck egg-white powder were improved by desugarization. Glucose oxidase and catalase of 31.24 and 781 units/mL, respectively, were added and then incubated at 30 ºC for 6.55 h prior to spray drying to inhibit the off-flavor and brown color [7]. Among the wide research on the application of glucose oxidase, there are only a few studies applied in desugaring egg white [7,8]. Moreover, no published study has explored the effects of glucose oxidase in desugaring to inhibit browning reaction in the hard-boiled egg white.

To examine the influence of process parameters on a change in food qualities, the isothermal kinetics model is often used, especially in thermal processing. Many studies confirm that using the general rate law of reaction combined with the Arrhenius equation is applicable to reasonably

describe quality degradation associated with thermal processing and storage [9–11]. This research therefore aimed to develop an isothermal kinetic model to examine color change in hard-boiled egg white at high temperatures and to minimize the browning reaction in the sterilized egg white mixed with glucose oxidase.

2. Experimental methods

2.1 Sample preparation

Fresh whole eggs were daily purchased from a Betagro shop (Bangkok, Thailand). The liquid egg white was manually separated from the yolk. The pH of liquid egg white was then adjusted to 9.0±0.05 with 0.1 M HCl. The egg-white liquid (albumen) was divided into 2 parts: (1) the untreated sample and (2) the sample treated with glucose oxidase solution. The glucose oxidase solution was prepared by adding 2 mL of 50-mM sodium acetate buffer (pH 5.1) into glucose oxidase (Sigma-Aldrich Corp., USA) and was kept in a refrigerator at 4 ºC. The enzyme solution of 48 µL was added to 8-mL egg white to obtain a treated sample with 240 units of glucose oxidase. This method was adapted from [7]. The mixture was subsequently stirred at 30 rpm in an incubator shaker (New Brunswick Innova 42/42R Shaker, Germany) at 30 °C for 6.5 hours. The lag time before reaching the steady-state temperature was minimized by using the sample holders (50 mm in diameter and 20 mm high) made of aluminum, which has high thermal conductivity (206 W/m•K) and equipped with an inserted 0.5-mm diameter thermocouple wire (T-type) to measure the surface temperature of the sample as shown in Figure 1. The treated and untreated samples of 8 mL each were filled into sample holders and sealed with lids and gaskets. The samples were heated at 70 ºC for 30 minutes in a water bath to solidify the egg white prior to cooling in ice water to minimize the thermal effect. The temperature used is higher than the coagulation temperature of egg whites in the range of 60–65 ºC. The sample holders were left at the ambient temperature to reach equilibrium before further thermal treatment.

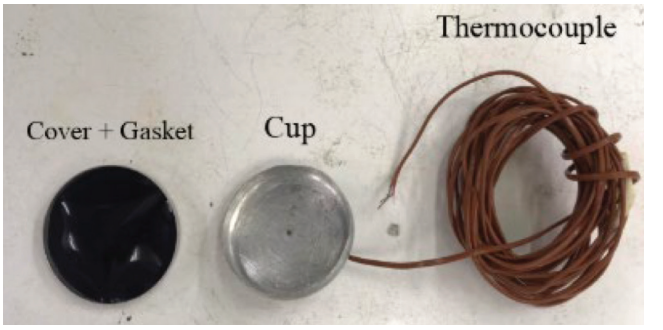


Figure 1 Sample holder equipped with a thermocouple wire.

2.2 Thermal treatment

The untreated and treated samples in 5 holders each were heated at various temperatures (100, 110, and 130 ºC) using a thermostatic oil bath (14 liters) containing ethylene glycol (Memmert-ONE, Bangkok, Thailand). A 2 kg dead weight was placed on the sample holder lid to ensure that the holders were hermetically sealed. Temperatures of the samples and the heating medium were recorded using a data logger (YOKOGAWA FX112-4-2, China). At an interval heating time of 5, 10, 15, 20, and 30 minutes, one sample was randomly taken and immediately cooled in ice water to cease the reaction. The experiments at each temperature were conducted in triplicate.

2.3 Color measurement

After thermal treatment, the color at the surface of the sample was measured by a spectrophotometer (Color Quest XE, Hunter Associates Laboratory, Inc., USA), equipped with a standard illumination source D65, the observation at 2° of the viewing field, and 1 inch of port size. The color determination for each treatment was carried out at five different positions, and then the average value was presented in terms of L^*, a^*, b^*, and yellowness index (YI), which is calculated according to Eq. (1) [12].

$$YI = 100 \times \frac{1.2985X - 1.1335Z}{Y} \quad (1)$$

where X, Y, and Z are the CIE tristimulus values.

2.4 Isothermal kinetic modeling

The kinetic model of color change is described by the yellowness index ratio, which is defined as the fraction of the yellowness index at a given time, $YI(t)$, to the initial yellowness index (YI_0)

$$YI_R = \frac{YI(t)}{YI_0} \quad (2)$$

For an isothermal reaction, the rate of YI_R change was assumed to follow the zero-and first-order kinetics, as shown in Eq. (3) and (4):

Zero-order reaction (n=0): $YI_R = YI_{R0} - k_T t$ (3)

First-order reaction (n=1): $YI_R = YI_{R0} e^{-k_T t}$ (4)

where YI_R is the yellowness index ratio at a given time, YI_{R0} is the initial index ratio, $k_T t$ is the rate constant, which is temperature dependent, t is time, and n is the reaction order. The temperature-dependent rate constant is assumed to follow Arrhenius's equation.

$$k_T = k_0 e^{-E_a/RT} \quad (5)$$

where k_0 is a pre-exponential factor, E_a is an activation energy, R is the universal gas constant, and T is the absolute temperature of the product surface, which is time-dependent. The constant parameters (k_0 and E_a) were determined by linear regression analysis.

However, the rate constant must be determined at a constant temperature for an isothermal reaction. The heating system was designed to approach the assumed isothermal condition by using a large volume of heating medium to obtain a constant temperature, high thermal conductivity of the sample holder, and the surface temperature of the sample arriving at the third time constant ($t=3\tau$), which elapses 95% of a full temperature response. In other words, the isothermal temperature for further study in the kinetic model is assumed when the temperature reaches 95% of the steady-state temperature that can be calculated according to Eq. (6) [13].

$$T = T_2 + (T_1 - T_2)e^{-t/\tau} \quad (6)$$

where T is the surface temperature of the product at a given time (t), T_2 is the temperature of the heating medium, T_1 is the initial temperature, and τ is a time constant.

The goodness of the fit between the predicted values and the observed values was calculated in terms of the coefficient of determination (R^2) and the root mean square error (*RMSE*) using Microsoft Excel® software.

$$R^2 = 1 - \sum_{i=1}^{N} \frac{(y_i - \hat{y}_i)^2}{(\hat{y}_i - \bar{y})^2} \quad (7)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N} (y_i - \hat{y}_i)}{N}} \quad (8)$$

where y_i is the ith observed value, $\hat{y}_i$ is the corresponding predicted value, $\bar{y}$ is the average of observed values, and N is the number of observations.

3. Results and discussion

3.1 Surface Temperature

Figure 2 shows the surface temperature of hardened egg white with/without glucose oxidase. During heating in the oil bath at 100, 110, and 130 °C, the surface temperature of both sample treatments rapidly rose from the initial temperature of 25 °C to 90, 100, and 120 °C,

respectively, and then stabled at those temperatures below the oil temperatures (5–10 °C) throughout the experiments. In this study, a heat-up period or a delayed time was inevitable because a large sample size was used so that the surface color of the sample was measurable. To compensate for the heat-up period of each experiment (i.e., 3–10 min), the constant temperatures used in the kinetic model were obtained from Eq. (6), which were 92, 98, and 115 °C for the oil bath temperatures of 100, 110, and 130 °C, respectively.

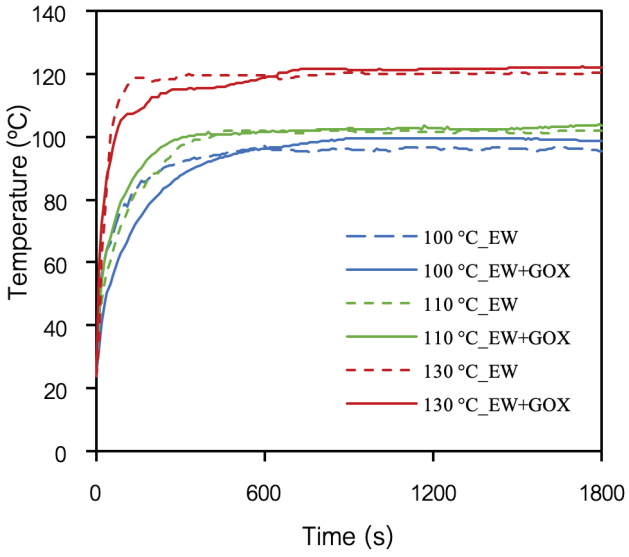


Figure 2 Surface temperatures of egg white with and without glucose oxidase undergoing heating at 100, 110, and 130 ºC oil temperatures.

3.2 Color Kinetics

Figures 3(a)(b)(c) show the instrumental values in terms of lightness (L^*), redness (a^*), and yellowness (b^*) of untreated egg whites. It was found that the L* of the samples steadily decreased with time from 91 initial value to 89, 87, and 75, when heated at 100, 110, and 130 ºC, respectively. It can be clearly observed that the L^* value at 130 ºC changed at a higher rate than in other cases. In addition, the L^* value remained constant during the first 5–10 min of the heating time, which was defined as the lag time or the heat-up period. The extent of the lag time was inversely proportional to the heating temperature. Without a change in L^* value, the browning reaction might not have started yet; this evidence was also observed in the cases of a^* and b^* values. The a^* value was initially negative (–2.78) and dramatically changed to be positive (+3.63) after heating at 130 ºC for 15 min when the surface temperature reached 121 ºC indicating that the color changed from green to red due to the temperature effect. The initially positive b^* (+7) indicates that the natural color of hardened egg white is pale yellow and increased to 10, 17, and 33 after heated at 100, 110, and 130 ºC, respectively. The influence of a time-temperature could be clearly observed. Particularly at a high temperature the substrates

of the Millard reaction, reducing sugar and amino acids, collide with each other more frequently leading to a faster reaction to generate the brown color of 4-hydroxy-5-methyl-3(2H)-furanone [4]. In other words, the browning reaction intensity could be indicated by a reduced L^* value and increased a^* and b^* values, accelerated by a high temperature.

An alternative way to present the color development is the use of yellowness index ratio. Figure 3(d) represents the yellowness index ratio of the hardened egg white surface treated with glucose oxidase compared with egg white without any enzymatic treatment. In all treatment conditions, the YI-ratio of desugarized egg white showed less than that of the untreated egg white. The YI-ratio of the sample heated at 100, 110, and 130 °C increased from the initial value of 1 to 1.5, 2.6, and 5.9, respectively, while the YI-ratio of egg white treated with glucose oxidase increased from 1 to the final values of 1.05, 1.13 and 2.56 when heated at 100, 110, and 130 °C, respectively. Obviously, the YI-ratio of the treated sample significantly reduced ($p<0.05$), especially at temperatures higher than 100 ºC.

Figure 4 displays the color development on the surface of egg white treated/untreated with glucose oxidase during heating at 100, 110, and 130 ºC. It appears that at 100 ºC the color of treated samples slightly changed throughout the 30-minute heating, while pale yellow color started to be observed after heating at 110 ºC for 30 minutes. At 130 ºC, it was noticeable that the pale-yellow color was observed within the first 10 minutes, and its intensity obviously increased with increasing heating time. For the hardened egg white surface treated with glucose oxidase, the brown color was not found for the samples of 100 and 110 ºC, but not the case of 130 ºC where the brown color could be observed at the first 10 minutes at which the surface temperature reached 119 ºC. The final brown color of the treated sample (EW with GOX) seems less than that of the sample without glucose oxidase (EW), but at a high temperature for a long time (130 °C for 30 min), the brown color was still occurred likely because of the extant presence of glucose in egg white. The observed results were consistent with the instrumental measurement.

Table 1 shows the estimated parameters of both zero-and first-kinetic models. Based on high R^2 and low $RMSE$, the first-order kinetic model (R^2 = 0.92–0.95 and $RMSE$ = 0.11–0.29) gives a better fit compared with the zero-order kinetic model (R^2 = 0.83–0.94 and $RMSE$= 0.16–0.3). The observed rate constants (k_T) of the YI-ratio increased with the treatment temperatures. For untreated samples, the k_T varied in the range of 2.98–10.2x10^{-4} s^{-1} based on the first-order reaction, whereas 8.29–24.7x10^{-5} s^{-1} for treated samples. Thus, the reaction rate was slowed down to around 10 folds. The E_a values were 62.93 and 152.42 kJ/mol for untreated and treated samples, respectively, which agree with the E_a of a non-enzymatic browning reaction (23–238 kJ/mol) [14].

The higher activation energy (E_a) indicates that the reaction is more difficult to occur. Moreover, the predicted yellowness index ratio obtained from the first-order reaction had good agreement with the observed data (R^2>0.9) as shown in Figure 5. The observed rate constants (k_T) were reduced by 10 folds approximately when the samples were treated with glucose oxidase, whereas the activation energies were increased.

4. Conclusion

The change of yellowness index (YI) of egg white undergoing a high-temperature treatment followed the first-order reaction. An increase in the activation energy (E_a) of desugared egg white indicated the high energy needed to initiate the reaction and the reaction rate was relatively slow down indicated by a low-rate constant. In conclusion, it could be confirmed that glucose oxidase could reduce the brown color development in egg white undergoing heating at high temperatures. This research demonstrated the potential of using glucose oxidase to reduce brown color development in shelf-stable egg-white products, which are processed at a high temperature.

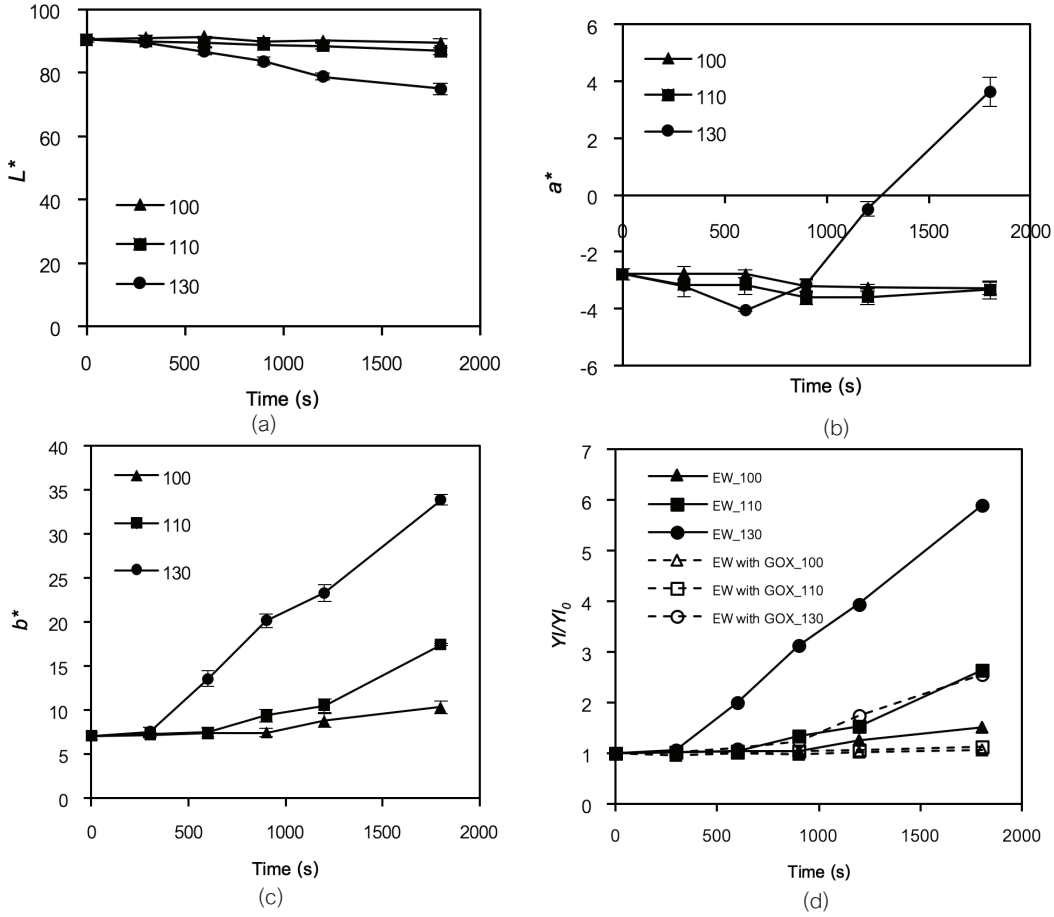


Figure 3 Changes in (a) lightness (L^*), (b) redness (a^*), (c) yellowness (b^*), and (d) yellowness index ratio of egg white during heating at different temperatures.

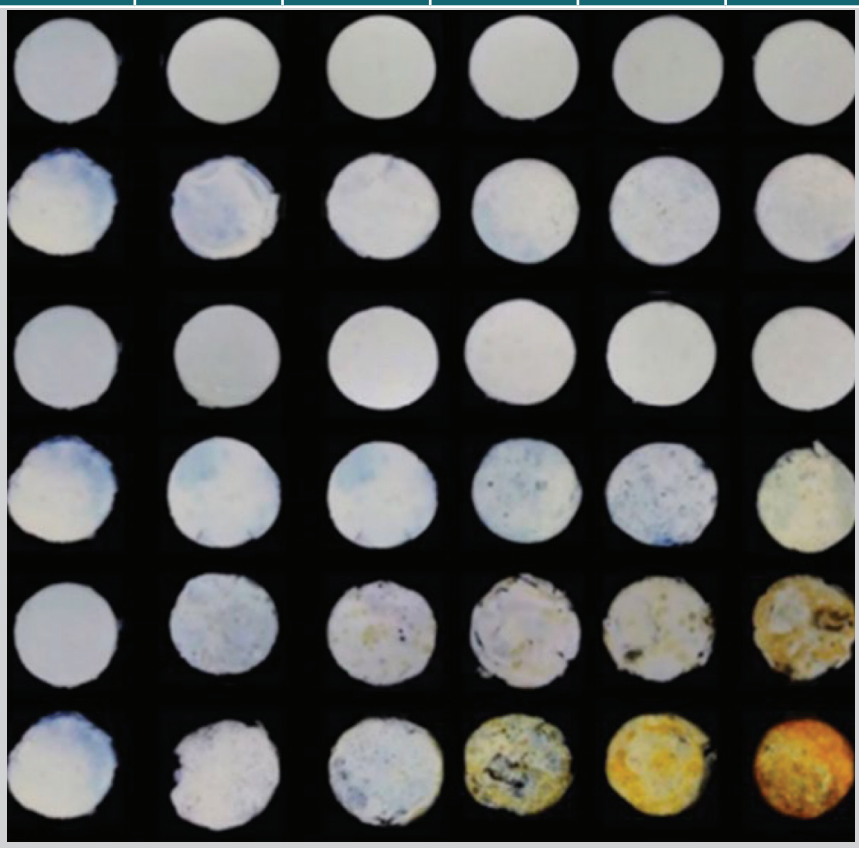
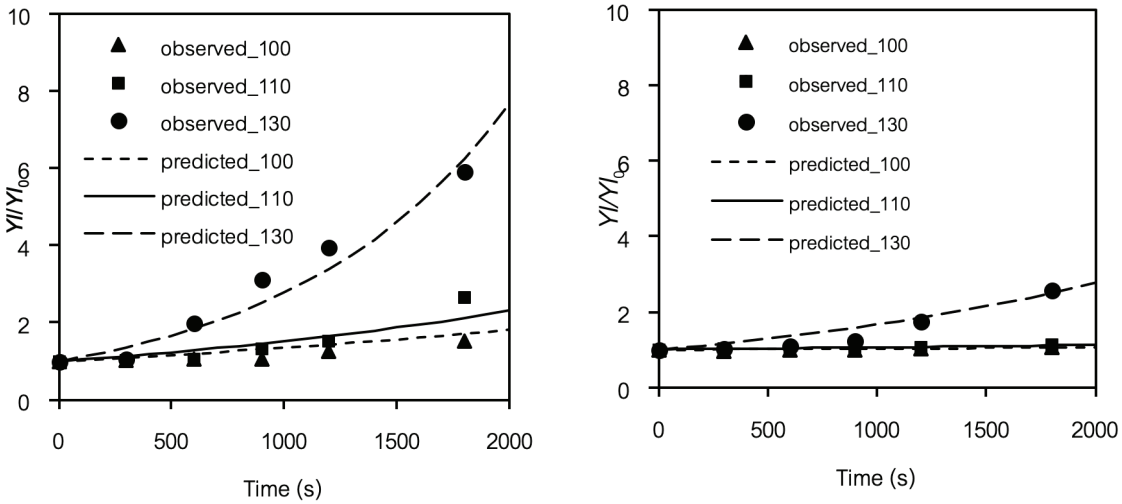
Oil temperature (°C)		Time (minute)					
		0	5	10	15	20	30
100	EW with GOX						
	EW						
110	EW with GOX						
	EW						
130	EW with GOX						
	EW						

Figure 4 Browning development on the surfaces of egg white (EW) and egg white with glucose oxidase (EW with GOX) during heating at 100, 110, and 130 °C for 5, 10, 15, 20, and 30 minutes.



(a) (b)

Figure 5 Comparison of the observed and predicted yellowness index ratio (YI/YI_0) obtained from the first-order kinetic model of (a) untreated egg white and (b) and treated egg white with glucose oxidase.

Table 1 Estimated zero-and first-order isothermal kinetic parameter for egg white (EW) and egg white with glucose oxidase (EW with GOX)

Parameter.		Zero order	First order
EW	k_{92} (s^{-1})	$3.27x10^{-4}$	$2.98x10^{-4}$
	k_{98} (s^{-1})	$5.71x10^{-4}$	$4.17x10^{-4}$
	k_{115} (s^{-1})	$2.51x10^{-3}$	$1.02x10^{-3}$
	k_0 (s^{-1})	$2.84x10^{11}$	$3.00x10^{5}$
	E_a (kJ/mol)	104.43	62.93
	R^2	0.9382	0.9449
	RMSE	0.2952	0.2878
EW with GOX	k_{92} (s^{-1})	$1.83x10^{-5}$	$8.29x10^{-5}$
	k_{98} (s^{-1})	$5.09x10^{-5}$	$2.72x10^{-5}$
	k_{115} (s^{-1})	$7.80x10^{-4}$	$2.47x10^{-4}$
	k_0 (s^{-1})	$5.84x10^{22}$	$1.76x10^{17}$
	E_a (kJ/mol)	165.75	152.42
	R^2	0.8298	0.9145
	RMSE	0.1621	0.1133

5. Acknowledgement

The authors gratefully acknowledge the National Science and Technology Development Agency (NSTDA) for their financial support through the FEPS scholarship.

6. References

[1] Lechpattanaporn K, Wang Y, Wang J, Tang J, Hallberg LM, Dunne CP. Sterilization of scrambled eggs in military polymeric trays by radio frequency energy. J Food Sci. 2005;70(4):E288–E294.

[2] Pastoriza S, Quesada J, Rufián-Henares JA. Lactose and oligosaccharides: Maillard reaction. Reference Module in Food Science [Internet]. 2018 [cited 2023 Jan 5];1-8. Available from: https://doi.org/10.1016/B978-0-08-100596-5.22552-3

[3] Zhang W, Tang J, Liu F, Bohnet S, Tang Z. Chemical marker M2 (4-hydroxy-5-methyl-3 (2H)-furanone) formation in egg white gel model for heating pattern determination of microwave-assisted pasteurization processing. J Food Eng. 2014;125:69-76.

[4] Bornhorst ER, Tang J, Sablani SS, Barbosa-Cánova GV. Development of model food systems for thermal pasteurization applications based on Maillard reaction products. LWT-Food Sci Technol. 2017;75:417-24.

[5] Llave Y, Takemori K, Fukuoka M, Takemori T, Tomita H, Sakai N. Analysis of browning of broiled foods by noncontact techniques: A case study for Japanese eggplant (*Solanum melongena*). J Food Process Eng. 2017;40(1):e12347.

[6] Scott D. Food stabilization, glucose conversion in preparation of albumen solids by glucose oxidase-catalase system. J Agric Food Chem. 1953;1(11):727-30.

[7] Quan TH, Benjakul S. Impacts of desugarization and drying methods on physicochemical and functional properties of duck albumen powder. Dry Technol. 2019;37(7):864-75.

[8] Sisak C, Csanádi Z, Rónay E, Szajáni B. Elimination of glucose in egg white using immobilized glucose oxidase. Enzyme Microb Technol. 2006;39(5):1002-7.

[9] Bali OD, Mittal GS. Kinetics of tofu color changes during deep-fat frying. LWT-Food Sci Technol. 2003;36(1):43-8.

[10] Matsuda H, Llave Y, Fukuoka M, Sakai N. Color changes in fish during grilling–Influences of heat transfer and heating medium on browning color. J Food Eng. 2013;116(1):130-7.

[11] Llave Y, Fukuda S, Fukuoka M, Shibata-Ishiwatari N, Sakai N. Analysis of color changes in chicken egg yolks and whites based on degree of thermal protein denaturation during ohmic heating and water bath treatment. J Food Eng. 2018;222:151-61.

[12] ASTM International. Standard practice for calculating yellowness and whiteness indices from instrumentally measured color coordinates. Paint-tests for chemical, physical, and optical properties. ASTM E313-05. West Conshohocken, PA : ASTM International; 2005.

[13] Dunn WC. Temperature and Heat. In: Introduction to instrumentation, sensors, and process control. Norwood, Massachusetts: Artech House Inc; 2006.p.149–155.

[14] Boekel MAJS van. Kinetic aspects of the Maillard reaction: a critical review. Food/ Nahrung. 2001;45(3):150–9.