

Contents lists available at ScienceDirect

Journal of Food Engineering

journal homepage: www.elsevier.com/locate/jfoodeng



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



Wenjia Zhang, Juming Tang*, Fang Liu, Stewart Bohnet, Zhongwei Tang

Department of Biological Systems Engineering, Washington State University, Pullman, WA 99164-6120, United States

ARTICLE INFO

Article history:
Received 12 April 2013
Received in revised form 25 August 2013
Accepted 17 October 2013
Available online 24 October 2013

Keywords: Microwave-assisted pasteurization (MAP) Egg white Gel model Chemical marker Heating pattern

ABSTRACT

Microwave-assisted pasteurization (MAP) is a potential thermal processing technology in which the non-uniform heating presents a challenge. This study evaluated the application of a chemical marker M2 (4-hydroxy-5-methyl-3(2H)-furanone) in an egg white gel model on the determination of possible heating patterns in prepackaged foods during MAP processing. The gel model samples were prepared by heating a homogeneous liquid egg white mixture (25% egg white, 1% D-ribose, 0.5% L-lysine) at 70 °C for 30 min. The chemical marker M2 formation was studied by heating the gel model samples in 75, 80, 85, 90, 95, and 100 °C oil bath for 5, 10, 15, 20, and 30 min. The marker yields were determined using high-performance liquid chromatography (HPLC). The color values of the heat-treated samples were measured using CIE $L^*a^*b^*$ and RGB models. The stability of M2 was evaluated at storage temperatures of 4 and 22 °C for 1, 3, 5, and 9 days. In order to validate the application of the new gel model system, the heating patterns of the gel models and marker yields of samples taken from 5 different locations of the MAP-processed gel models at 75 and 100 °C were analyzed. Results showed that the M2 formation in egg white increased linearly with heat treatment time at 75-95 °C, while a slight concavity was observed for samples treated at 100 °C. Color parameters L^* and G values were found to be significantly correlated with the heating temperatures. During storage, the M2 retention rate decreased with increasing time and temperature, while samples treated for longer times were more stable. Salt addition had no significant effect on the M2 yield within the studied time-temperature combination. The color change of egg white gel models due to different M2 yield after the MAP process could be clearly recognized using a computer vision method.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

Microwave-assisted thermal processing is a promising food processing technology that utilizes microwave power as a heating source to inactivate microbes to produce safe prepackaged foods (Ohlsson, 1991; Salazar-González et al., 2012). The fast volumetric heating from the inside of the food overcomes the disadvantage of slow heating rates produced in conventional thermal processes. Therefore, the processing time can be significantly reduced to retain better product quality (Vadivambal and Jayas, 2010).

A major challenge in the development of microwave-assisted thermal processes is the non-uniform heating (Keefer and Ball, 1992; Tang et al., 2008; Koskiniemi et al., 2011). In order to achieve certain microbial lethality, it is essential to ensure that the cold spots of the food (which receives the least amount of heat) be adequately heated. Since it is impractical to use temperature sensors

for multi-point temperature monitoring during the process, a chemical marker method was developed at the United States Army Natick Research Center to map the heating pattern and locate the cold spots of foods (Kim and Taub, 1993). This method is based on the Maillard reaction between amino acids and reducing sugars. in which various chemical compounds can be produced (Hodge. 1953). Three chemical markers, namely, 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one(M-1), 4-hydroxy-5-methy-3 (2H)-furanone(M-2) and 5-hydroxymethylfurfural(M-3), were identified as time-temperature indicators formed from different reactants under various conditions. These three chemical markers have been used to determine the heating uniformity in ohmic heating (Kim et al., 1996), aseptic processing (Ramaswamy et al., 1996), radio frequency processing (Wang et al., 2004), and microwave-assisted thermal sterilization (MATS) (Prakash et al., 1997; Lau et al., 2003; Wang et al., 2004, 2009; Pandit et al., 2006).

However, the heat distribution determination using chemical marker analysis by high-performance liquid chromatography (HPLC) is expensive and time consuming, while the reliability of

^{*} Corresponding author. Tel.: +1 509 335 2140; fax: +1 509 335 2722. E-mail address: jtang@wsu.edu (J. Tang).

the computer simulation results needs validation (Resurreccion et al., 2013). A computer vision method was developed by Pandit et al. (2007a,b) for rapid visualization of the heating patterns in food after a particular heating process. The method is based on correlating the color value of the gel model (mashed potato with chemical marker M2) in gray scale with thermal lethality F₀ and M2 yield. All the studies above focused on the sterilization process, in which the product temperature exceeds 110 °C. For microwaveassisted pasteurization (MAP) process, in which the product temperature is lower than 100 °C, the model food systems used for MATS system including whey protein gels (Wang et al., 2004, 2009) and mashed potatoes (Pandit et al., 2006) cannot be applied due to their high gelation temperatures and low processing temperature of MAP. Therefore, new model food systems need to be developed for heating pattern determination of MAP process. In a recent study, Zhang et al. (2013) explored the use of egg white as a suitable gel model for pasteurization applications. In order for it to be used as an indicator of heat distribution, certain chemical marker precursors need to be added to it as color agents, so that color change of the model system can be detected after processing using the computer vision system. Among the three identified chemical markers, M2 is formed by rearrangement of the Amadori compound by 2, 3-enolization after the reaction between reducing sugar and amino acid when the environmental pH is higher than 4.5 (Kim et al., 1996). It is thus suitable to be used in the alkaline condition of egg white mixture. Moreover, the yield of M2 can be relatively high to ensure enough color change especially when using ribose as a reactant. Ashoor and Zent (1984) reported that among the different amino acids they studied, L-lysine exhibited a high tendency toward Maillard browning. Therefore, in this study, M2 was chosen as the possible chemical marker and D-ribose and L-lysine as the reactants to reflect heat distribution in the egg white gel model system for the MAP process.

The objectives of this study were to investigate the formation of chemical marker M2 in egg white gel model when using D-ribose and L-lysine as reacting chemicals under pasteurization conditions, in order to correlate the color change with processing temperature, and to validate the application of the new gel model system in heating uniformity evaluation of the MAP process using a computer vision method. Since salt needs to be added to the gel model system to obtain different dielectric properties to model various foods (Zhang et al., 2013), the effect of salt addition on the M2 yield was also investigated. Moreover, the stability of the chemical marker M2 during storage was studied to understand the effect of storage time and temperature on the accuracy of the heating pattern results using the computer vision method.

2. Materials and methods

2.1. Sample preparation

In order to produce homogeneous egg white gel model with enough gel strength, commercial "Just Whites" all natural egg white powder (0% total fat, 2% sodium; Deb-El Foods Corporation, Elizabeth, NJ) was used to prepare the liquid egg white mixtures with a 25% solid content. A predetermined amount of egg white powder was mixed with 35 °C double deionized (DDI) water on a magnetic stirrer for 3 min. The mixture was then kept in a 35 °C water bath for 20 min for further rehydration. Chemical marker precursors, D-ribose and L-lysine, were chosen at concentrations of 1% and 0.5%, due to their reaction ratio of 2:1 and a lysine content ranging from 1.1% to 1.6% in heated and unheated egg albumin at a 25% solid content (Boctor and Harper, 1968). The chemicals were added to the liquid mixture when it was cooled to room temperature. The mixture was then stirred for 1 h to ensure

uniform distribution of the chemical reactants in the liquid matrix. The foam formed on top of the mixture during stirring was removed before the homogeneous mixture was filled and sealed in custom-built aluminum thermal kinetics testing (TKT) cells (Fig. 1) designed at Washington State University. The TKT cells have an inner diameter of 50 mm and an inner height of 5 mm, in order to minimize the come-up-time (CUT, time needed for the sample temperature to achieve the processing temperature) and to get enough samples for color measurement after the heat treatments. The samples in TKT cells were heated in a 70 °C water bath for 30 min and cooled in ice water immediately to form gel models. The samples were equilibrated to room temperature prior to the oil bath treatments.

2.2. Oil bath treatment

In order to cover a likely range of time-temperature combinations for MAP processing, the egg white gel models were kept sealed in the TKT cells and heated in an oil bath (oil was replaced by ethylene glycol as the heating medium) at 75, 80, 85, 90, 95, and 100 °C. A 0.1 mm diameter type-T (copper-constantan) thermal couple (Omega Engineering, Stamford, CT) was inserted through the top lid to 1 mm under the surface of the gel model to monitor the sample temperature during heating. The CUT, which was defined as the time for the sample temperature to reach 0.5 °C below the set temperature, was around 2–2.5 min. Timing was started after the CUT. After each heating time interval of 5, 10, 15, 20, and 30 min, the heating cells were taken out of the water bath and cooled immediately in ice water to minimize the thermal effect during cooling.

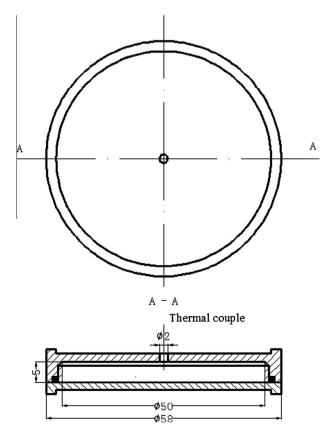


Fig. 1. Schematic diagram of the custom-built aluminum thermal kinetics test (TKT) cell.

2.3. Marker yield analysis using HPLC

The egg white gel model samples were taken out of the TKT cells and equilibrated to room temperature. A sample weight 0.2 g was taken out precisely from the region at surface of each gel sample around the thermal couple sensor tip for HPLC analysis. The gel sample was ground in 2 mL extraction buffer (10 mM $\rm H_2SO_4)$ using a glass mortar and pestle. After grinding, the extraction mixture was transferred into a 2 mL plastic centrifuge tube and centrifuged at 14,000 rpm for 10 min using an Eppendorf centrifuge (Eppendorf AG, Hamburg, Germany). The supernatant was collected and filtered through a 0.45 μm PTFE syringe filter (Pall Corporation, Port Washington, NY), and then sealed in a C4011-1w glass HPLC sample vial (National Scientific Company, Rockwood, TN).

The M2 yield was determined using an Agilent 1100 HPLC system (Agilent Technology, Santa Clara, CA) equipped with a diode array detector. 0.25 μL of each marker sample was injected into the HPLC system by an automatic injection system and flowed through a 100×7.8 mm fast acid analysis column (Bio-Rad Laboratories, Hercules, CA) with 10 mM H_2SO_4 mobile phase at a rate of 1 mL/min. The detecting wavelength was set at 285 nm (Kim and Taub, 1993). An M2 standard curve was obtained by running commercial M2 (Sigma-Aldrich Co. LLC, St. Louis, MO) solutions prepared at different concentration levels (Fig. 2) by the same HPLC procedure. The marker yield (mg marker per g of sample) was calculated as:

$$Marker\ yield = \frac{Peak\ area}{55,235} \times \frac{Volume\ of\ extract\ (2\ mL)}{Sample\ weight\ (g)} \tag{1}$$

2.4. Color value determination

A computer vision system (CVS) was used for the sample color analysis (Fig. 3). The CVS consisted of an EOS D60 digital camera (Canon Inc., Melville, NY), a 910-20 Copystand (Bencher, Inc., Antioch, IL) as sample and camera stand, an ALZO 300 Table Top Studio with 24" Riser Platform and 2 "Cool Lites" (Akces Media LLC, Bethel, CT) as lighting system, and a desktop computer with image analysis software. The lights were amounted on both sides of the sample with a height of 100 cm. The digital camera was mounted downwardly at a height of 120 cm above the sample stand. The lights of the lighting system were turned on 15 min before each image taking process to warm up the light bulbs for consistent light intensity. The fresh and oil bath treated gel model samples

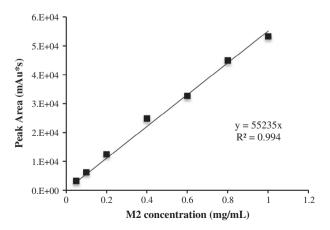


Fig. 2. Standard curve of chemical marker M2 (4-hydroxy-5-methyl-3(2H)-furanone) in 10 mM sulfuric acid buffer.

(diameter = 50 mm, height = 5 mm) were taken out of the TKT cells and placed on a black background.

According to Yam and Papadakis (2004), among the three mostly used color models (CIE $L^*a^*b^*$, RGB, and CMYK), $L^*a^*b^*$ color parameters are independent of the input or output devices, while RGB colors are device dependent but most closely resemble the way that human eyes perceive color. Therefore, the $L^*a^*b^*$ and RGB color parameter values were used to study the correlation between color values and treatment temperatures. In the $L^*a^*b^*$ model, L^* stands for luminance or lightness component, while a^* and b^* are chromatic parameters which stand for green to red and blue to yellow, respectively. In the RGB model, the R, G, and B values stand for the intensity of the three primary color spectrum of red, green and blue, respectively (León et al., 2006). On each sample image, the color values of 3 points were obtained using the histogram tool in CS6 Photoshop Software (Adobe system, Inc., San Jose, CA) to represent the color of the whole sample.

2.5. Effect of salt addition on M2 yield

A predetermined amount of salt was added to the liquid egg white mixtures prepared as mentioned above to a final salt addition of 0, 50, 100, or 200 mM. The liquid mixtures were filled and heated in the TKT cells at 70 °C for 30 min to form gel models. The gels were then heated in a 90 °C oil bath for 10, 20, or 30 min. Samples without salt addition were used as a control for each treatment. The chemical marker M2 yield in the heated gel samples were then determined using the HPLC method as mentioned above.

2.6. Storage stability of M2

The egg white gel model samples heat-treated at 85 °C for 5, 10, 15, 20, and 30 min were used to study the effects of storage time and temperature on the stability of M2 formed in egg white gel model. The marker samples were extracted following the same extraction procedures as mentioned above, sealed in glass HPLC vials, and stored at 4 °C and 22 °C in dark for 1, 3, 5, and 9 days. The chemical marker M2 concentrations were monitored during storage after each storage time interval. The storage ability of M2 was represented by the retention rate value, which was calculated as:

Rentention rate =
$$\frac{Cs_0 - Cs}{Cs_0} \times 100\%$$
 (2)

where Cs_0 is the M2 concentration of freshly treated samples, and Cs is the M2 concentration of the sample after storage.

2.7. Microwave-assisted pasteurization (MAP) treatment

8 oz Egg white liquids (without salt addition) were filled in to 10 oz plastic trays (14 cm \times 9.5 cm \times 3 cm) and heated in a 70 °C water bath for 30 min to form the egg white gel models. After heating, the gel models were cooled at 4 °C and then vacuum-sealed in 8-oz plastic pouches (16 cm \times 12 cm) for MAP treatments. The single mode 915 MHz MATS (Microwave-Assisted Thermal Sterilization) system developed at Washington State University was used to validate the application of the new gel model system at pasteurization temperatures. The MATS system consisted of pre-heating, microwave heating, holding, and cooling sections. In order to ensure the consistence of the process, the MATS system was warmed up for 10 min before running. After warming up, the temperature of circulating water inside the system was set up to obtain desired process temperatures (75 and 100 °C, which were respectively the lowest and highest temperatures used in the oil bath treatment, monitored at the inlet of the microwave heating cavity). The gel

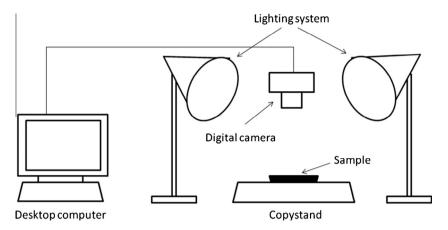


Fig. 3. Components of the computer vision system for sample color and heating pattern analysis.

models in pouches were placed on the food package conveyor belt and loaded to the pre-heating section (30 °C) for pre-conditioning with hot water. After 20 min pre-heating, the pouches were moved through the microwave heating cavities with a conveyor speed of 40 in./min. The microwave power outputs of each cavity were set at 2.5 kW, 3.1 kW, 1.5 kW, and 1.4 kW for desired process temperature of 75 °C, or 4.6 kW, 6.4 kW, 2.5 kW, and 2.4 kW for 100 °C. The sample temperature was rapidly increased by both microwave power and circulating water. The gel models were then moved to a holding section with circulating water of 75 or 100 °C. A cooling section was followed where the samples were cooled down using tap water (25 °C) and unloaded from the system.

2.8. Heating pattern analysis for the MAP processed gel models

According to the former experimental data (Tang et al., 2008) and computer simulation results (Resurreccion et al., 2013), the cold spots of MATS system were always in the middle layer. Therefore, the MAP processed gel models were cut horizontally in the middle. The middle layer images of the gel models were taken using the computer vision system described above. The software for heating pattern analysis (Pandit et al., 2007) included a CS6 Photoshop (Adobe system, Inc., San Jose, CA) and an IMAQ vision builder software (National Instrument Product, Austin, TX). The analysis was based on a script built in the IMAQ software developed by Pandit et al. (2007b).

2.9. Statistical analysis

The marker yield, color values, and kinetic parameters were obtained from replicated measurements using which proved the proposed method of using Excel (Microsoft Corporation, Redmond, WA) and were shown as Means \pm Standard deviation. The version 14.1 Minitab software (Minitab Inc., State College, PA) was used to obtain the correlation coefficients and ANOVA tests with a significance level of P = 0.05.

3. Results and discussion

3.1. M2 formation

The chemical marker M2 yields in egg white gel models treated at 75, 80, 85, 90, 95 and 100 °C for 5, 10, 15, 20, and 30 min are shown in Fig. 4. The marker yield increased with both heating time and temperature. Within 30 min of heat treatment, the chemical marker M2 yield increased linearly at temperature of 75–95 °C ($R^2 > 0.98$). In the review by van Boekel (2001) on kinetics aspects

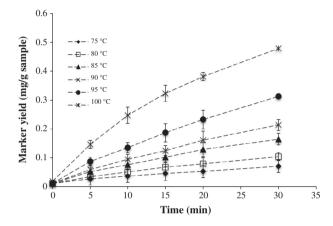


Fig. 4. Chemical marker M2 yield in egg white gel models heat-treated at various pasteurization temperatures (n = 4).

of Maillard reaction, the formation of the relatively more stable intermediate and advanced Maillard reaction products (MRP) were reported to follow zero-order kinetics, which was the same as shown in our results at 75–95 °C. The reason could be that the amounts of these MRPs were usually low when compared to the concentrations of the reaction precursors. However, when the treatment temperature increased to 100 °C, a slight concavity was observed on the curve showing a deviation from zero order kinetics. Some researchers have reported that the formation of M1 in broccoli (Kim and Taub, 1993) and M2 in whey protein gels (Lau et al., 2003) and mashed potatoes (Pandit et al., 2006) followed first order kinetics at sterilization temperatures (116, 121, 126, and 131 °C). It could be inferred that with the increase of processing temperature, the larger amount of M2 production began to become a limiting factor which reduced the reaction rate.

3.2. Correlation between color parameters and temperature

The correlation coefficients between color parameters of the heat treated samples (including L^* , a^* , b^* , and R, G, B values) and temperatures (75–100 °C) at each treatment time of 5–30 min are summarized in Table 1. For all time intervals, significant negative correlations were found between the temperatures and color parameters L^* and G values (P < 0.05). L^* represents the luminance of the sample in the $L^*a^*b^*$ color system, while the G value represents the green color in the RGB model. The negative correlations indicated that after a certain time of heat treatment, the luminance and greenness of samples processed at higher temperatures were

Table 1 Correlation between heat treatment temperature and color values L, a, b, and R, G, B of egg white gel models (n = 3).

Treatment time (min)	Correlation coefficient (r)					
	L	а	b	R	G	В
5	-0.900*	-0.956*	-0.163	-0.765	-0.932*	-0.818
10	-0.905^{*}	-0.945^{*}	-0.339	-0.832	$-0.900\degree$	-0.927^{*}
15	-0.949^*	0.620	-0.457	-0.930^{*}	-0.953^{*}	-0.907^{*}
20	-0.988^*	0.568	-0.534	-0.977^*	-0.971^*	-0.936^{*}
30	-0.983^{*}	0.854	-0.584	-0.988^*	-0.967^{*}	-0.946^{*}

^{*} P < 0.05.

less intense. Combining with the result that the marker yield increased with heat treatment temperature, a negative correlation between the L^* and G values with marker yield can be deduced, which indicates that the locations on processed gel models with higher L^* or G values receives lower amount of thermal energy. Therefore, for a gel model processed using the MAP system, it is possible to analyze its heating pattern using certain statistical software to locate the cold spots as locations with the highest L^* or G values.

3.3. Effect of salt on M2 yield

Salt addition was used to adjust the dielectric loss factor of the egg white gels to model various foods with different dielectric properties (Zhang et al., 2013). The effect of salt addition on the M2 yield in egg white gel model is shown in Fig. 5. No significant difference was found for samples with different salt addition (0–200 mM) and heated at 90 °C for 0, 10, 20, and 30 min (P > 0.05). The results agreed well with the report of Pandit et al. (2007) that salt addition of 1% did not affect the yield of M2 in mashed potato when heated at 121 °C. Thongraung and Kangsanan (2010) also reported that the addition of salt (0.5–2.5%, w/v) inhibited the formation of final stage Maillard reaction products but showed no significant effect on the intermediate Maillard reaction products. It can be concluded that salt addition will not change the heating pattern results of the egg white gel models.

3.4. Stability of M2 during storage

The stability of M2 (extracted from egg white gel models heated at 85 °C) represented by the retention rate during storage at 4 °C and 22 °C are shown in Fig. 6a and b, respectively. The retention rate of M2 decreased with storage time for samples stored at both temperatures. Since M2 is an intermediate product of the Maillard reaction (Kim and Taub, 1993), it was possible that a certain

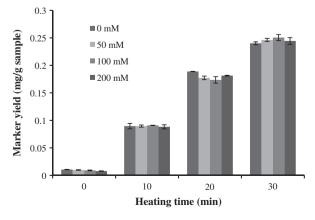
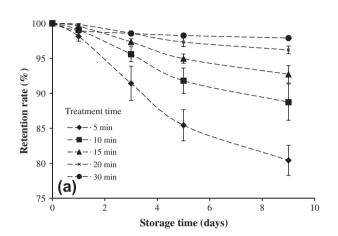


Fig. 5. Effect of salt addition on the M2 yield in egg white gel model when heated in $90 \,^{\circ}$ C water bath for 0, 10, 20, and $30 \, \text{min} \, (n = 4)$.



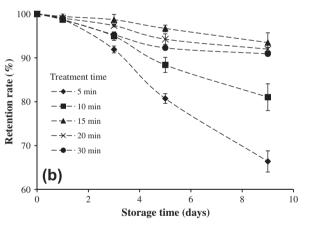


Fig. 6. Retention rate of chemical marker M2 (extracted from egg white gel model samples treated at 85 °C for various times) during storage at 4 °C (a) and 22 °C (b) for 1, 3, 5, and 9 days (n = 4).

amount of the M2 was transformed into other chemical compounds under the storage conditions.

The effect of storage temperature on the M2 retention rate was also significant. At a storage temperature of $22\,^{\circ}$ C, the decrease of M2 retention rate was more significant than that at $4\,^{\circ}$ C. After 9 days of storage at $4\,^{\circ}$ C, the retention rate of M2 extracted from the sample treated for 5 min decreased to 80.4%, while that of the same sample stored at $22\,^{\circ}$ C decreased to 66.4%. The result suggested that the degradation or transformation of M2 into other compounds was favored at higher storage temperatures.

The retention rate of M2 was also affected by the heat treatment time. After a certain storage time, the M2 retention rate for samples heat-treated for longer times were higher than those treated for shorter time intervals. As shown in Fig. 6a, after 9 days of storage at 4 °C, the retention rate of M2 extracted from the sample heat treated for 5 min decreased to 80.4%, while that of the sample heat-treated for 30 min was 97.9%. The reason for the difference could be the much higher original M2 concentrations in the

samples heat-treated for longer times before storage. A similar phenomenon was found for samples stored at 22 °C. The retention rate of the 5 min treated sample decreased to 66.4%, while that of the samples treated for 15, 20, and 30 min ranged from 90.9-93.5% without significant difference (P > 0.05).

It can be concluded from the result that, in order to ensure the accuracy and reliability of the chemical marker method, the storage time and temperature after the processing of the gel model must be carefully controlled. For microwave processing at higher temperatures and for longer times, the gel models can stay relatively stable after storage at 4 °C for a few days. For example, M2 from the gel models heated at 85 °C for 20 and 30 min could be considered as stable after storage at 4 °C for up to 9 days since the M2 retention rates were higher than 95%. However, for processes at lower temperatures or for shorter times, it is essential to make sure the marker yield or heating pattern analysis is carried out shortly after the microwave process.

3.5. Validation of M2 application in the MAP process

The color changes of gel model samples in trays after MAP process at 75 and 100 °C were analyzed using computer vision system and the results were shown in Fig. 7a and b. The figures were used to differentiate the color change of different locations due to the accumulated M2 production from various time-temperature profiles. Since the temperature at different locations were not monitored, no temperature-indicating scale was included. The differences between heating patterns shown in Fig. 7a and b were due to the different processing temperatures and microwave power setups.

Based on the application of computer vision method on the heating pattern determination of MATS system, the parts of the gel model in red color in the images were defined as hot regions (hot spots) which received the highest amount of thermal energy during the processes, whereas the parts in blue color were cold regions (cold spots) which have received the lowest amount of thermal energy. Other colors between blue and red described the

regions which have received medium amount of thermal energy. In order to ensure that the computer vision method results are also reliable for the new model system for MAP process, the color parameters L* and G values and the M2 yield of samples taken from the MAP-processed egg white gel models (from locations 1-5 as shown in Fig. 7a and b) were determined. The results of gel models MAP-processed at 75 and 100 °C are shown in Figs. 8 and 9, respectively. As shown in Fig. 8a, the marker yield of locations 1, 2, and 3 for 75 °C processed gel model were close due to their low concentration level, while those of locations 4 and 5 were much higher and location 5 showed the highest M2 yield. It could be concluded that locations number 5 absorbed the highest amount of heat during the process, followed by location 4 and then the other three. Therefore, location 5 can be concluded as the hot spot for 75 °C processed gel model. The L^* and G values of the same sample decreased from locations 1-5, which agreed with our findings that the L^* and G values negatively correlated with the process temperature. Furthermore, it can be concluded that the computer vision method had a higher sensitivity than the marker yield analysis by HPLC. For gel model samples MAP-processed at 100 °C, the marker yield also increased from locations 1–5, while the L^* and G values decreased. A linear relationship was found between marker yield with both L^* and G values (Fig. 9), which proved the proposed method of using L^* and G values for cold/hot spot determination.

In order to further validate the cold or hot spots, a mobile metallic ELLAB sensor was used to monitor the temperatures of cold/hot spots determined from the heat distribution results obtained by using computer vision system. The ELLAB sensors installed inside a protective metal tube with 2 mm diameter and 50 mm length (Luan et al., 2013) was inserted into the horizontal middle layer of the egg white gel model at the cold and hot spots predetermined for a 90 °C process. The samples in trays were then MAP-processed at microwave heating and holding temperatures of 90 °C. Results showed that the temperatures of hot spots were all higher than those of the cold spots, which well verified the cold and hot spot locations. With the assistance of chemical marker M2 and egg white gel model system to locate the cold and hot

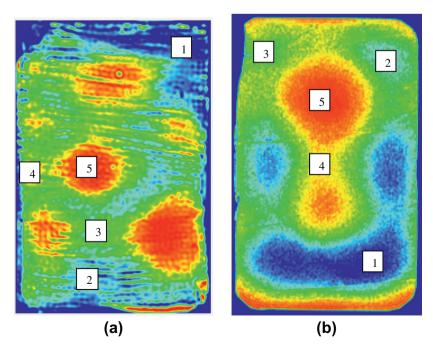


Fig. 7. Heating pattern results of egg white gel models (middle layer) with chemical marker M2 after microwave-assisted pasteurization processing in 915 MHz single mode microwave system at 75 °C and 100 °C (location number 1: Blue, 2: Aqua, 3: Green, 4: Yellow, 5: Red). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

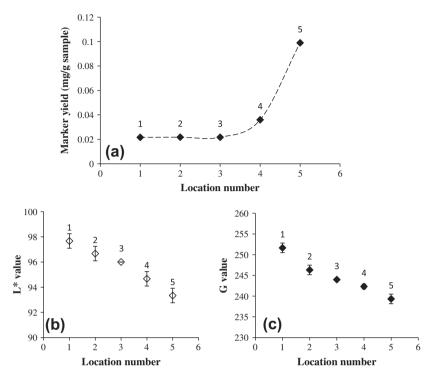


Fig. 8. Chemical marker M2 yield (a) and color parameters L* (b) and G values (c) of egg white gel models after microwave-assisted pasteurization process at 75 °C (n = 4).

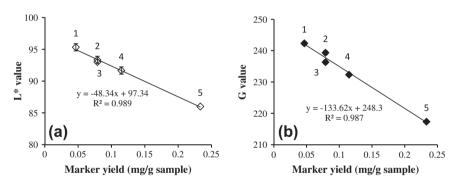


Fig. 9. Correlation between chemical marker M2 yield and color parameters G and L^* values of egg white gel model after microwave-assisted pasteurization process at 100 °C (n = 4).

spots, the temperatures of the cold and hot spots could be monitored for calculation of required thermal lethality or quality parameters to develop safe, reliable, and uniform microwave assisted pasteurization processes.

4. Conclusions

The formation of chemical marker M2 in egg white gel models at pasteurization temperatures of 75–100 °C increased with both time and temperature. The retention rate of M2 samples extracted from the heat-treated egg white gel models decreased with increasing storage time. The retention rate of samples stored at 4 °C was much higher than that at 22 °C. Samples treated at higher temperatures or for longer times could stay stable during 4 °C storage. However, for the more mild microwave-assisted pasteurization processes, it is recommended to analyze the gel model samples shortly after the process. With the addition of 1% D-ribose and 0.5% L-lysine, the egg white gel models at the central cut by MAP-process at temperatures higher than 75 °C could clearly show the heating pattern by the computer vision method. The negative

correlation between the L^* and G color values with the treatment temperatures suggests a possibility of using statistical methods to locate the cold spots of the gel models.

Acknowledgments

This project was supported by the Agriculture and Food Research Initiative of the USDA National Institute of Food and Agriculture, Grant Number #2011-68003-20096. The authors also thank the Chinese Scholarship Council for providing a scholarship to Wenjia Zhang for her Ph.D. studies at Washington State University.

References

Ashoor, S.H., Zent, J.B., 1984. Maillard browning of common amino acids and sugars. Journal of Food Science 49, 1206–1207.

Boctor, A.M., Harper, A.E., 1968. Measurement of available lysine in heated and unheated foodstuffs by chemical and biological methods. Journal of Nutrition 94 289-296

Hodge, J.E., 1953. Dehydrated foods: chemistry of browning reactions in model systems. Journal of Agricultural and Food Chemistry 1 (15), 928–943.

- Keefer, R.M., Ball, M.D., 1992. Improving the final quality of microwavable foods. Microwave World 13 (2), 14–21.
- Kim, H.J., Taub, I.A., 1993. Intrinsic chemical markers for aseptic processing of particulate foods. Food Technology 47 (1), 91–97, 99.
- Kim, H.J., Taub, I.A., Choi, Y.M., Prakash, A., 1996. Principles and applications of chemical markers of sterility in high-temperature-short-time processing of particulate foods. In: Lee, T.C., Kim, H.J. (Eds.), Chemical Markers for Processed and Stored Foods. American Chemical Society, Washington, DC, pp. 54–69.
- Koskiniemi, C.B., Truong, V.D., Simunovic, J., McFeeters, R.F., 2011. Improvement of heating uniformity in packaged acidified vegetables pasteurized with a 915 MHz continuous microwave system. Journal of Food Engineering 105, 149–160.
- Lau, H., Tang, J., Taub, I.A., Yang, T.C.S., Edwards, C.G., Mao, R., 2003. Kinetics of chemical marker formation in whey protein gels for studying high temperature short rime microwave sterilization. Journal of Food Engineering 60 (4), 397– 405
- León, K., Mery, D., Pedreschi, F., León, J., 2006. Color measurement in $L^*a^*b^*$ units from RGB digital images. Food Research International 39, 1084–1091.
- Luan, D., Tang, J., Pedrow, P.D., Liu, F., Tang, Z., 2013. Using mobile metallic temperature sensors in continuous microwave assisted sterilization (MATS) systems. Journal of Food Engineering 119, 552–560.
- Ohlsson, T., 1991. Microwave processing in the food industry. European Food and Drink Review 7, 9–11.
- Pandit, R.B., Tang, J., Mikhaylenko, G., Liu, F., 2006. Kinetics of chemical marker M-2 formation in mashed potato—a tool to locate cold spots under microwave sterilization. Journal of Food Engineering 76 (3), 353–361.
- Pandit, R.B., Tang, J., Liu, F., Mikhaylenko, G., 2007a. A computer vision method to locate cold spot in foods during microwave sterilization processes. Pattern Recognition 40 (12), 3667–3676.
- Pandit, R.B., Tang, J., Liu, F., Pitts, M., 2007b. Development of a novel approach to determine heating pattern using computer vision and chemical marker (M-2) yield. Journal of Food Engineering 78 (2), 522–528.
- Prakash, A., Kim, H.J., Taub, I.A., 1997. Assessment of microwave sterilization of foods using intrinsic chemical markers. Journal of Microwave Power and Electromagnetic Energy 32 (1), 50–57.

- Ramaswamy, H.S., Awuah, G.B., Kim, H.J., Choi, Y.M., 1996. Evaluation of a chemical marker for process lethality measurement at 110 °C in a continuous flow holding tube. Journal of Food Processing and Preservation 20 (3), 235–249.
- Resurreccion, F.P., Tang, J., Pedrow, P., Cavalieri, R., Liu, F., Tang, Z., 2013. Development of a computer simulation model for processing food in a microwave assisted thermal sterilization (MATS) system. Journal of Food Engineering 118, 406–416.
- Salazar-González, C., Martín-González, M.F.S., López-Malo, A., Sosa-Morales, M.E., 2012. Recent studies related to microwave processing of fluid foods. Food and Bioprocess Technology 5, 31–46.
- Tang, Z., Mikhaylenko, G., Liu, F., Mah, J.H., Tang, J., Pandit, R., Younce, F., 2008. Microwave sterilization of sliced beef in gravy in 7-oz trays. Journal of Food Engineering 89 (4), 375–383.
- Thongraung, C., Kangsanan, S., 2010. Influence of pH, NaCl and pre-incubation on utilisation of surimi wash water in generation of antioxidative material by using the Maillard reaction. International Journal of Food Science and Technology 45 (8), 1696–1702.
- Vadivambal, R., Jayas, D.S., 2010. Non-uniform temperature distribution during microwave heating of food materials—a review. Food and Bioprocess Technology 3, 161–171.
- Van Boekel, M.A.J.S., 2001. Kinetic aspects of the Maillard reaction: a critical review. Nahrung 45 (3), 150–159.
- Wang, Y., Lau, M.H., Tang, J., Mao, R., 2004. Kinetics of chemical marker M-1 formation in whey protein gels for developing sterilization processes based on dielectric heating. Journal of Food Engineering 64 (1), 111–118.
- Wang, Y., Tang, J., Rasco, B., Wang, S., Alshami, A.A., Kong, F., 2009. Using whey protein gel as a model food to study the dielectric heating properties of salmon (Oncorhynchus gorbuscha) fillets. LWT–Food Science and Technology 42, 1174– 1178.
- Yam, K.L., Papadakis, S.E., 2004. A simple digital imaging method for measuring and analyzing color of food surfaces. Journal of Food Engineering 61 (1), 137–142.
- Zhang, W., Liu, F., Nindo, C., Tang, J., 2013. Physical properties of egg whites and whole eggs relevant to microwave pasteurization. Journal of Food Engineering 118, 62–69.