

Regular Paper

Browning Behavior of Calcium Maltobionate-water System

(Received May 21, 2023; Accepted July 21, 2023)

(J-STAGE Advance Published Date: July 25, 2023)

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Abstract: The purpose of the present study was to clarify the browning behavior of calcium maltobionate (CaMb). CaMb samples with varying water content (0–50 %) and water activity (0–0.98) were prepared. Absorbance due to the browning of CaMb-water samples increased linearly with an increase in holding time, and the browning rate was evaluated as a pseudo-zero-order reaction at each temperature (353–413 K). The effect of temperature on the browning rate of CaMb samples was analyzed using the Arrhenius formula, and the pre-exponential factor and activation energy were determined. In addition, the browning rate at 298 K (typical ambient condition) was determined according to the Arrhenius behavior. The browning rate at 298 K slightly decreased, markedly increased, and then decreased with an increase in water content and water activity. This behavior can be explained by the monolayer effect, plasticizing effect, and dilution effect of water molecules. There was a linear relationship between the natural logarithm of the pre-exponential factor and activation energy according to the thermodynamic compensation rule. The effect of water content on the activation energy was fitted using the cubic function. From these results, an approach for prediction of the browning of CaMb during thermal condensation was established.

Key words: calcium maltobionate, browning, water activity, activation energy, TG-DTA

INTRODUCTION

Calcium maltobionate (CaMb), which consists of glucose and gluconic acid, is a calcium-based material with practical applications to food and pharmaceutical products. For example, CaMb has high water-solubility as well as Ca absorption-promoting and bone density-improving effects.^{1,2,3} In addition, CaMb has a high glass to rubber transition temperature (T_g) and a substantial resistance to crystallization.^{4,5} Thus, CaMb is anticipated to be not only an effective source of Ca, but also a physical modifier for food products.

In our previous study, the effect of CaMb-addition on the physical properties of hard candies was investigated.⁶ From the result, it was found that the T_g of normal sugar candy was lower than the expected value. In addition, the enhancement of red and yellow colors (browning) was observed. This suggests that CaMb itself and/or sugars affected by CaMb hydrolyze and produce color pigments during heat-concen-

tration at a high temperature. However, there is no quantitative data on the browning of CaMb. The purpose of this study, thus, was to clarify the browning behavior of CaMb as a function of temperature, time, and water content.

MATERIALS AND METHODS

Materials. Calcium carbonate and maltose monohydrate were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). Catalase and glucose oxidase were purchased from Amano Enzyme Inc. (Aichi, Japan). CaMb powder was prepared according to our previous studies.^{4,5} The purity of CaMb was confirmed to be > 99 % by high-performance liquid chromatography (HPLC).

Sample preparation. CaMb samples with varying water content were prepared within the water content range of 0 and 50 % (w/w), at intervals of approximately 10 % water content. To prepare the aqueous solution samples, CaMb powder was dissolved in distilled water, and adjusted to 50 and 40 % water content. In addition, the sample with 50 % water content was vacuum-dried under ambient conditions, and the water content was adjusted to 30 % based on the weight loss. In our previous studies,^{4,5} a water sorption isotherm of CaMb at 298 K was established, as shown in Fig. S1 (see J. Appl. Glycosci. Web site). The water sorption isotherm was obtained using the Guggenheim, Anderson, and de Boer (GAB) model,

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Abbreviations: CaMb, Calcium maltobionate; T_g , glass to rubber transition temperature; W , water content; a_w , water activity; k , browning rate; k_{298K} , calculated browning rate at 298 K; k_0 , pre-exponential factor for browning; E_a , activation energy for browning.

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$$W = \frac{W_m C K a_w}{(1 - K a_w)(1 + (C - 1) K a_w)} \quad (\text{Eq. 1})$$

where W and a_w are the equilibrium water content and water activity, respectively. The constants W_m , C , and K were determined to be 6.45 (g-H₂O/100g-solid), 9.19, and 0.954, respectively. According to the GAB parameters, the CaMb samples with water contents of 50, 40, and 30 % were evaluated as $a_w = 0.98, 0.95, \text{ and } 0.89$, respectively.

To prepare the low-water content samples, CaMb powder was vacuum-dried at 353 K for 8 h, and the fully dried sample was regarded as the anhydrous sample ($a_w = 0$).^{4,5} In addition, the anhydrous sample was equilibrated at 298 K for longer than 7 days in a desiccator with saturated NaCl ($a_w = 0.753$) and Mg(NO₃)₂ ($a_w = 0.529$), and the water content was adjusted to 19.1 and 10.7 %, respectively.⁴

Thermal treatment, browning, and chemical composition. The aqueous solution samples (1 mL) and low-water content samples (0.5 g) were hermetically enclosed in a 20-mL glass vial ($\phi = 27$ mm). The samples were put in an oven preset at 353, 373, 393, and 413 K for a maximum of 12 weeks. After the thermal treatment, distilled water was added to the vial, and absorbance (ABS) at 480 nm was measured at an optical path length of 1 cm using a UV-visible spectrophotometer (Biospec-1600; Shimadzu Co., Kyoto, Japan).⁷ The ABS value was divided by the solute content of the sample solution.

To confirm the chemical composition, a 50 % (w/w) CaMb solution (1 mL) was held at 100 °C for up to 36 h, and the composition was investigated using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD).⁶ HPAEC-PAD was carried out on a DX-500 Carbohydrate system (Dionex Co., Sunnyvale, CA, USA) equipped with a Dionex CarboPac PA-1 (250 × 4.0 mm) in combination with a CarboPac PA-1 guard column (50 × 4.0 mm). The column was eluted at 303 K with 100 mM NaOH at 1.0 mL/min. The composition was evaluated using a gradient of IM sodium acetate in 100 mM NaOH (0 mM for 0–5 min and 0–200 mM for 5–35 min). Glycerol was employed as an internal standard.

Thermal condensation treatment and browning. Thermal condensation treatment of CaMb samples was carried out using thermogravimetry-differential thermal analysis (TG-DTA; DTG-60, Shimadzu Co., Kyoto, Japan). The temperature of the equipment was calibrated with indium. The sample (43–58 mg) was put into an aluminum pan, and then thermal condensation was carried out. The thermal condensation conditions (heating rate, target temperature, and holding time at the target temperature) are listed in Table 1. Following the treatment, the sample was dissolved in distilled water, and ABS was evaluated as explained earlier.

Table 1. Measurement conditions for TG-DTA.

No.	Heating rate (K/min)	Temperature (K)	Holding time (min)
1	0.5	413	0
2	1	413	0
3	2	413	0
4	2	373	0.5
5	2	393	0.5
6	2	413	0.5

RESULTS AND DISCUSSION

Browning of CaMb-water mixtures under a hermetic isothermal condition.

The effect of holding time on the ABS of CaMb-water samples heated at 373 K is shown in Fig. 1. The ABS increased linearly with an increase in holding time because of the progress of browning. For reference, images of the heated CaMb samples are shown in Fig. S2 (see J. Appl. Glycosci. Web site). The linear proportion in the time course of ABS is similar to that for non-enzymatic browning of freeze-dried solids.^{8,9,10,11} According to previous studies,^{8,9,10,11} the progress of browning was assumed as a pseudo-zero-order reaction, and the browning rate constant (k , 1/h) was determined from the slope in the time course of ABS.

The effect of temperature on the k of CaMb samples was analyzed using the Arrhenius formula (Eq. 2).

$$k = k_0 \exp\{-E_a / (R \cdot T)\} \quad (\text{Eq. 2})$$

where k_0 , E_a , R , and T are the pre-exponential factor (1/h), activation energy (J/mol), gas constant (J/(mol·K)), and temperature (K), respectively. The Arrhenius plot of the k for CaMb samples is shown in Fig. 2. From the intercept and slope in the Arrhenius plot, $\ln k_0$ and E_a were obtained. The values are listed in Table 2.

The E_a values of the CaMb samples ranged between 81.8 and 174.6 kJ/mol. The values were in good agreement with the E_a values (83.6–156.0 kJ/mol) for non-enzymatic browning between a reducing sugar and amino acid in a dehydrated amorphous matrix.^{10,11,12} From Fig. 2, k at 298 K (k_{298K}) was calculated as the browning reaction under ambient conditions. The effect of water content (% w/w) on k_{298K} is shown in Fig. 3A. The solid curve was obtained by fitting the cubic function to the experimental data. In addition, the water content of the approximate curve was converted to a_w through the GAB equation (Eq. 1), and the effect of a_w on k_{298K} is described in Fig. 3B. The k_{298K} slightly decreased, markedly increased, and then decreased with an increase in water content and a_w . This behavior is similar to the relation-

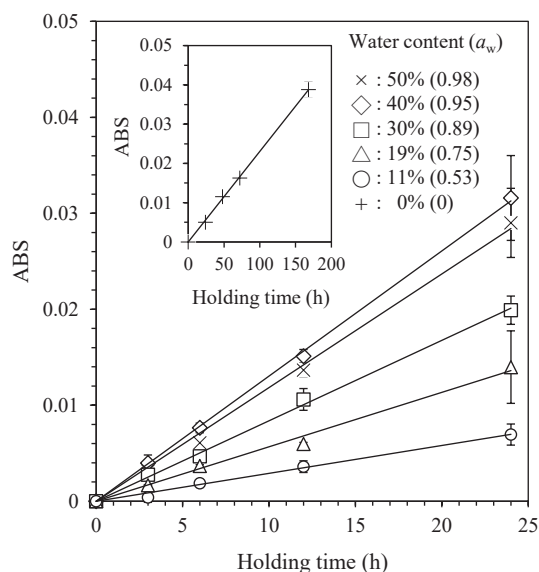


Fig. 1. Time course of the ABS of CaMb-water samples held at various temperatures.

Values are expressed as mean \pm SD ($n = 3$). Browning rate was determined as a pseudo-zero order-reaction rate.

ship between the non-enzymatic browning reaction rate and a_w .^{13,14} The effect of a_w on the k_{298K} of CaMb is discussed according to the interpretation for non-enzymatic browning as follows.

In a fully dehydrated condition, amorphous materials turn to a glassy state, and thus their macroscopic molecular mobility is apparently frozen. CaMb turns to a glassy state at a lower water content than 11.8 % and/or at a lower a_w than 0.56;⁴ the values are indicated by the vertical dotted line in Figs. 3A and B, respectively. The k_{298K} values of glassy

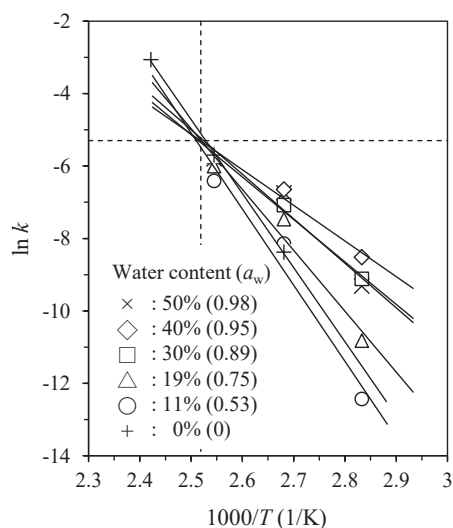


Fig. 2. Arrhenius plot for the browning rate of CaMB-water samples.

The vertical and horizontal dotted lines show T_{iso} (397.0 K) and $\ln k_{iso}$ (-5.297), respectively, for the CaMB-water system.

Table 2. Arrhenius parameters for the browning of CaMB samples with varying water content.

Water content (w/w)	a_w	E_a (J/mol)	$\ln k_0$
0	0	170.0	46.4
10.7	0.49	174.6	47.4
19.1	0.77	139.7	37.0
30.0	0.87	97.4	24.1
40.0	0.92	81.8	19.5
50.0	0.95	102.1	25.7

CaMB are low because of the highly limited molecular mobility. The k_{298K} of the anhydrous sample was slightly higher than that of the sample with 10 % water content (a_w 0.53). This can be explained by the stabilizing effect of the water monolayer. According to the a_w theory,^{13,14} the water monolayer covers the hydration sites on the surfaces of biomaterials; thus, access of chemical reactants to the surfaces of the biomaterials is diminished, and biomaterials covered by the water monolayer are more stable than anhydrous ones. Monolayer water content and monolayer a_w for CaMb are 6.06 % and 0.26, respectively.⁴ The values are indicated by the vertical dashed line in Figs. 3A and B, respectively. It is noted that the monolayer water content and monolayer a_w correspond to the minimum browning rate observed in the approximate curve.

At a water content higher than 11.8 % and/or at an a_w higher than 0.56, CaMb turns into a rubbery state, and molecular mobility markedly increases with an increase in water content and/or a_w because of the water plasticizing effect. Thus, k_{298K} largely increases with an increase in water content and/or a_w when CaMb is in the rubbery condition.

The k_{298K} showed a maximum value at around 40 % water content and/or a_w 0.95, and then slightly decreased. This behavior is explained by the dilution effect. The chemical reaction rate commonly decreases with the decrease in solute (reactants) content, as the frequency of collisions between reactants diminishes under a condition of excess water. In the case of non-enzymatic browning reaction, the amino group (e.g., amino acids) and carbonyl group (e.g., reducing sugar) commonly play the role of reactants. CaMb, on the other hand, undergoes electrolytic dissociation in the water system, and thus collisions between calcium ions and maltobionate ions will occur depending on the molecular mobility of the system.

To understand the browning process of CaMb, the chemical composition of a 50 % CaMb solution held at 373 K for up to 36 h was evaluated using HPAEC-PAD. A typical HPAEC-PAD chromatogram is shown in Fig. S3 (see J. Appl. Glycosci. Web site). A large peak due to maltobionate ions and a smaller peak due to glucose were observed. From the relative peak values, it was noted that maltobionate ions gradually decreased, and conversely, glucose increased with

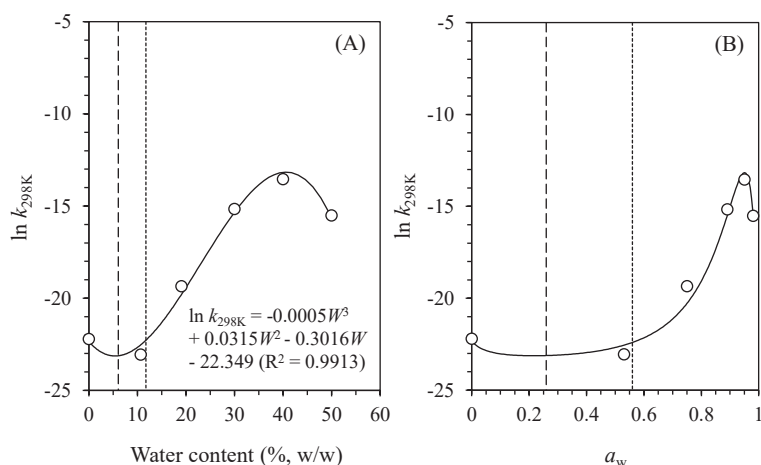


Fig. 3. Effect of water content (A) and a_w (B) on the calculated browning rate at 298 K.

The vertical dashed lines show monolayer water content (A) and monolayer a_w (B). The vertical dotted lines show water content (A) and a_w (B) at $T_g = 298$ K.

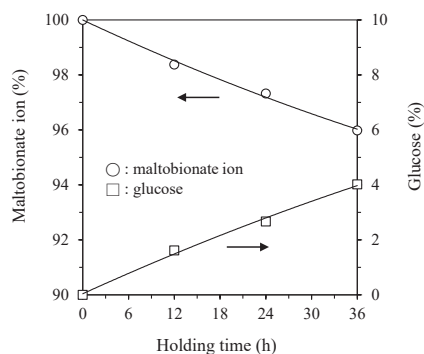


Fig. 4. Time course of the decomposition for the 50 % CaMB aqueous solution.

the increase in heating time (Fig. 4). This suggests that maltobionate ions decomposed into glucose and gluconate ions during heating.

Glucose itself is a typical source of browning. To confirm this, a 50 % glucose solution was heated at 373 K for 24 h, and the ABS value was evaluated to be 0.0029 ± 0.0009 ($n = 3$, mean \pm SD); the value was much lower than that of CaMb (0.0290 ± 0.0036), suggesting that browning of glucose is accelerated by gluconate and/or calcium ions. To obtain a more detailed understanding of this, the browning behavior of CaMb-glucose, gluconate ion-glucose, and calcium ion-glucose systems should be compared.

Browning of CaMb-water mixtures during thermal condensation.

In order to predict the browning of CaMb-water mixtures during the heat-condensation process, the effect of water content and/or a_w on $\ln k_0$ and E_a was analyzed. The pre-exponential factor can correspond to the activation energy according to the thermodynamic compensation rule¹⁵⁾¹⁶⁾ as follows,

$$\ln k_0 = a + b \cdot E_a \quad (\text{Eq. 3})$$

where a (dimensionless) and b (mol/kJ) are constants. From the b value (slope of the linear proportion), isokinetic temperature (T_{iso} , K) can be evaluated as follows,

$$T_{\text{iso}} = 1/(b \cdot R) \quad (\text{Eq. 4})$$

T_{iso} is the temperature at which the kinetic constant does not vary even when environmental factors (e.g., a_w , pH, and

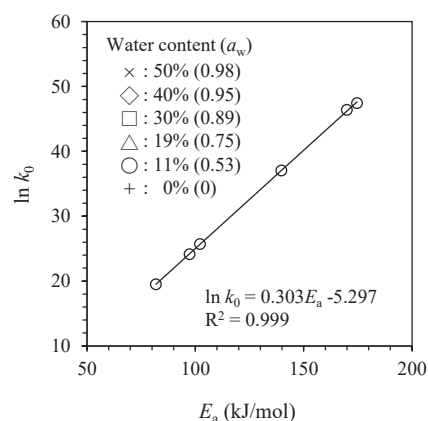


Fig. 5. The relationship between $\ln k_0$ and E_a for the browning of the CaMB-water system.

pressure) are changed.¹⁶⁾ The intercept (a) of the linear proportion, on the other hand, corresponds to the kinetic constant at T_{iso} ($\ln k_{\text{iso}}$).

The relationship between $\ln k_0$ and E_a is shown in Fig. 5. As expected, $\ln k_0$ increased linearly with an increase in E_a . From the linear proportion, T_{iso} and $\ln k_{\text{iso}}$ for the CaMb-water system were determined to be 397.0 K ($1,000/T_{\text{iso}} = 2.519$) and -5.297 , respectively. The values are indicated by the parallel and vertical dashed lines, respectively, in Fig. 2. It is noted that the T_{iso} and $\ln k_{\text{iso}}$ values were in good agreement in the crossover points provided by each Arrhenius line.

The effect of water content (%) on E_a for the browning of CaMb is shown in Fig. 6A. The solid curve described in Fig. 6A was obtained using the cubic function as an approximate curve. In addition, the water content of the approximate curve was converted to a_w through the GAB equation (Eq. 1), and the effect of a_w on E_a is shown in Fig. 6B. The E_a slightly increased, markedly decreased, and then increased with an increase in water content and a_w . These behaviors can be interpreted as being similar to the effect of water content and/or a_w on the $k_{298\text{K}}$ of CaMb (monolayer water, plasticizing, and dilution), as explained earlier.

From the cubic function describing the effect of water content on E_a , browning of the CaMb-water system during thermal condensation can be predicted as a function of

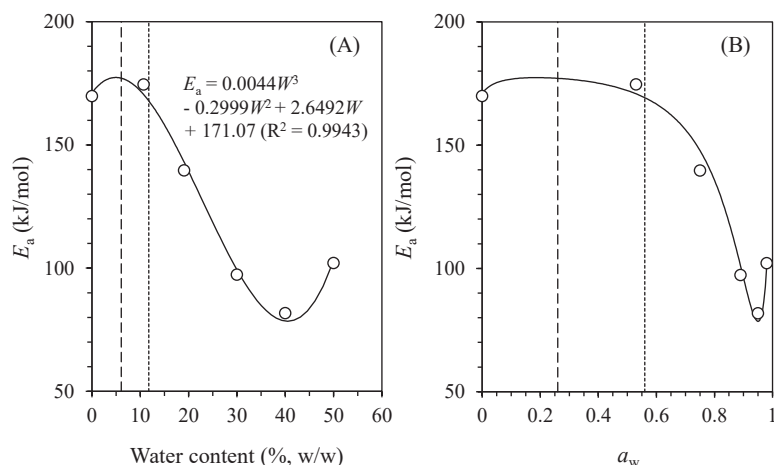


Fig. 6. Effect of water content (A) and a_w (B) on E_a for the browning of the CaMB-water system.

The vertical dashed lines show monolayer water content (A) and monolayer a_w (B). The vertical dotted lines show water content (A) and a_w (B) at $T_g = 298$ K.

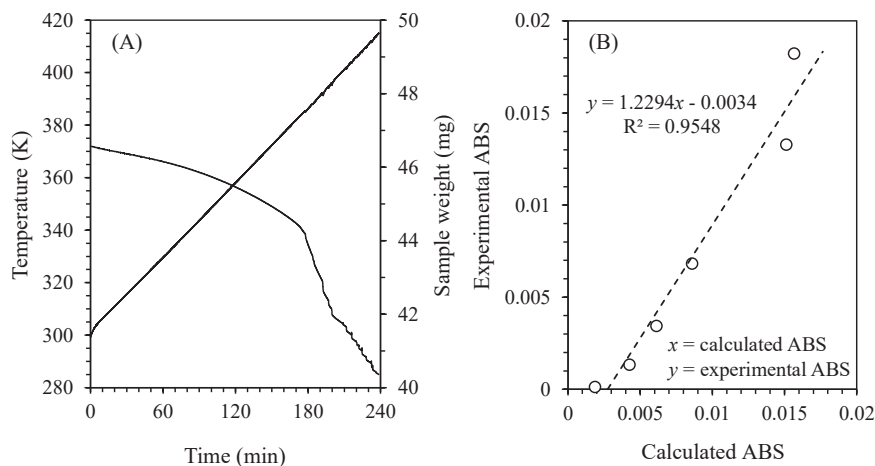


Fig. 7. Typical TG-DTA curve for CaMb with 19.1 % water content ($a_w = 0.753$) heated at 0.5 K/min up to 413 K (A) and the relationship between experimental ABS and calculated ABS for the thermally condensed CaMb (B).

temperature and water content. TG-DTA can simultaneously measure the changes in temperature and water content of a sample. To confirm this, TG-DTA and ABS measurements for CaMb with 19.1 % water content were carried out. A typical TG-DTA curve is shown in Fig. 7A. The sample weight gradually decreased and markedly decreased both below and above approximately 387 K. The sample weight was converted to water content at each temperature, accounting for the initial water content, and ABS was calculated according to Fig. 6A, Fig. 5, and Eq. 2. The relationship between experimental ABS values and calculated ABS values is shown in Fig. 7B. The predicted value was proportional to the experimental value with a minor deviation. This deviation between the calculated value and the experimental value could be attributed to the difference in thermal transfer between the TG-DTA system and the vial held in the oven.

CONCLUSION

Browning of the CaMb-water system was systematically examined in the present study. Our results are expected to be useful for predicting the behavior of the CaMb-water system during hermetic storage and thermal condensation. The origin of the browning was suggested to be the decomposition of maltobionate ions into glucose and gluconate ions. The browning of glucose is likely accelerated by the co-solutes (maltobionate, gluconate, and calcium ions) in the system. For a more detailed understanding, the browning behavior of CaMb-glucose, gluconate ion-glucose, and calcium ion-glucose systems should be further investigated in future studies.

CONFLICTS OF INTEREST

This study was financially supported by San-ei Sucochemical Co., Ltd. (Aichi, Japan) as part of a collaborative research project.

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