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Drying and Denaturation Kinetics of Beta-Lactoglobulin during Convective Drying

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ABSTRACT

Beta-Lactoglobulin (β -Lg) is the largest protein fraction of whey proteins comprising more than 65%. Being the major fraction, it determines many of the unique properties of whey proteins. Thus, the denaturation of β -Lg during drying can limit the application of whey proteins as ingredient. This study was carried out to measure and predict the drying and denaturation kinetics of β -Lg during drying at droplet level in a convective drying environment. The moisture content and temperature scenarios during the drying process were predicted using reaction engineering approach (REA)-based model. The REA-predictions were coupled with the first order reaction kinetics to predict the denaturation kinetics. Single droplets of β -Lg (10% w/v; 1.5 ± 0.1 mm initial diameter) were used throughout drying experiments carried out at two temperatures (65 °C and 80 °C) at a constant air velocity (0.5 m/s) for 10 minutes. There was a good agreement between the experimental data and the REA predictions. The model predictions fitted the experimental data with $\pm 5.70\%$ and $\pm 3.50\%$ error for moisture-time and temperature-time profiles, respectively. A combination of the REA-based model with first order kinetics model was also successful in predicting the denaturation kinetics.

Keywords: Beta-Lactoglobulin, denaturation kinetics, drying kinetics, single droplet drying, reaction engineering approach (REA).

1. Introduction

Whey proteins are widely used in food product formulations due to their nutritional and functional properties. Beta-Lactoglobulin (β -Lg) is one of the major components of whey proteins and it is largely responsible for imparting the unique gelation, foaming and emulsification properties to whey proteins. In addition, β -Lg also has very good flavour binding properties which makes it suitable ingredient in many food and beverage products (Jime'nez-Castano et al., 2005). Furthermore, β -Lg is a good source of amino acids and has high solubility and clarity over a broad pH range. These properties have enabled this protein to use as an active agent in various protein-fortified beverages, such as fruit juices and sports drinks (Ikeuchi, et al., 2008; Chatterton et al., 2006). An additional benefit associated with β -Lg is that it exhibits mild antioxidant activity which is reportedly due to the free thiol group present in the structure (Shpigelman et al., 2010). The molecular weight of β -Lg is 18.4 kDa and its structure contains 2 disulphide bridges and one free cysteine residue.

In our previous study of isothermal heat treatment (in aqueous medium) and convective air drying of whey protein isolate (WPI), we showed that β -Lg was more susceptible to heating and drying stresses compared to other major whey proteins such as Bovine Serum Albumin (BSA) (Haque et al., 2013a). About 70% of β -Lg was found to be denatured while 57% of the BSA was denatured when dried at 80 °C for 10 minutes. It is therefore a major concern at industry manufacturing and using milk protein powders since denatured and aggregated proteins have poor solubility in water and also have inferior emulsifying and other functional properties (Anandharamakrishnan et al., 2007). The functional properties of protein depends on its molecular composition and structure and the folded polypeptide chains perform protein's biological functions. External forces such as dehydration stresses can unfold the secondary and higher order structure of proteins and negatively affect their stability, especially in aqueous

systems. In the case of spray drying, high temperature and interfacial stresses are implicated to the denaturation of proteins (Haque, et al., 2015b). A more scientifically robust way of overcoming this problem is to carefully control the drying parameters during spray drying (Anandharamakrishnan et al., 2007). For this purpose, a clear understanding of the relationship between drying and denaturation kinetics is required. In particular, the changes brought about by the denaturing stresses have to be quantified in order to control the protein denaturation. Despite being major fraction of whey protein, information regarding behaviour or response of β -Lg molecules under stresses is very limited in the literature.

Spray drying is widely used technique in food and pharmaceutical industry as it is relatively economical compared to freeze drying and also it has considerably high throughput (Chavez & Ledebor, 2007; Haque & Adhikari, 2014). Despite the above mentioned advantages, a suitable technology for measuring drying and denaturation kinetics of protein droplets, *in situ*, in a spray drying chamber is yet to be developed. Due to the presence of billions of droplets in a spray drying chamber and also due to prevailing complex flow pattern, the relationship between drying and denaturation kinetics of β -Lg in spray drying process is not available. Hence, in absence of the *in situ* measurement technology, single droplet drying (SDD) of proteins is, so far, the most useful technology to measure both drying and denaturation kinetics at droplet level (Sano & Keey, 1982; Che et al., 2012; Haque et al., 2016). SDD also enables imaging of the surface topography of a drying droplet and hence enables acquiring the morphological data of drying droplets/particles. Therefore, the drying and denaturation kinetics, and surface morphology of β -Lg can be measured and interpreted using SDD. In this work, we present the drying and denaturation kinetics and surface morphology of single droplet of aqueous solution of β -Lg in particle formation process using SDD. We also present the prediction of the above mentioned data using reaction engineering approach (REA) tools. It is known that the spray drying system operates with two control temperatures; inlet temperature and outlet

temperature. The outlet temperature is very sensitive to product quality because atomized droplet contains less water toward the end of drying. The hot air comes at direct contact of the moist particles and causes damage to the particles. Hence, the protein powder manufactures as well as researchers are trying to reduce the outlet temperature of the drying chamber (from high temperature i.e. 80°C to low temperature i.e. 65°C or even lower) for minimal effect on the product. We used these two temperatures (80°C and 65°C) for drying the protein droplets to present a comparative pictures of denaturation of protein both at higher and lower drying temperatures. The findings, presented in this work, will enable the manufacturers to tailor and control the drying parameters to produce structurally intact and minimally denatured β -Lg powders through spray drying. The REA based modelling approach can also be used for dryer wide simulations by embedding the REA-based modelling into Computational Fluid Dynamics (CFD) environment (Lin & Chen, 2006; Patel & Chen, 2005). In this study, the lumped reaction engineering approach (L-REA) is implemented to model the average moisture content during drying.

2. Experimental Section

2.1. Materials

High purity ($\geq 95\%$) β -Lg was purchased from Sigma-Aldrich (New South Wales, Australia). The acetonitrile and Deuterium oxide (D_2O) were also purchased from Sigma-Aldrich (New South Wales, Australia). The trifluoroacetic acid (TFA) was purchased from Fisher Scientific (Victoria, Australia).

2.2. Methods

2.2.1. Single Droplet Drying (SDD)

The design, working principle and other details of this instrument are provided elsewhere (Adhikari et al., 2007). The aqueous 5 ± 0.1 μL droplets of β -Lg (10% w/v) solution were suspended on the tip of a glass filament of 0.2 ± 0.01 mm diameter. The β -Lg droplets which were suspended to the glass filaments were held inside the drying chamber by using a bored Teflon cylinder. The drying air was allowed to pass through a series of columns containing dried molecular sieves and dried silica gels. This dried air was finally passed through the drying chamber. Two drying temperatures (65°C and 80°C) were used and these temperatures were maintained using a PID controller. The relative humidity values of the drying air were measured by using EL-USB relative humidity data logger (Lascar Electronics, UK), and were found to be $2\pm0.5\%$ at these two drying temperatures. The wet bulb temperatures were 26°C and 30°C at air temperatures of 65°C and 80°C , respectively. The droplets were dried for 600s. The droplets were collected at every 100 s interval in order to measure the denaturation of β -Lg as a function of drying time and temperature. The velocity of air passing through the stationary droplet was maintained at 0.5 m/s in all the experiments reported. Loss of mass and temperature history were recorded in a computer. Images of the drying droplets were captured using a digital camera (EOS 60D, Canon, NSW, Australia) at $50\times$ magnification. The camera was attached to the back of the single droplet drying instrument and abled to focus in the drying chamber.

2.2.2. Determination of denaturation of β -Lg by using reversed phase (RP)-HPLC

The droplets dried to predetermined time using the SDD instrument were collected carefully in Eppendorf microtubes and diluted to $100\text{ }\mu\text{g/mL}$ with deionized water. The pH of this solution was then adjusted to 4.6 by adding 0.1M HCl that would separate the un-denatured and irreversibly denatured β -Lg (Parris & Baginski, 1991; Haque et al., 2015). After keeping

the β -Lg solution samples at this pH for 30 minutes, they were centrifuged at 13,000 rpm (11,500 g) for 10 minutes. The supernatant was collected and injected onto the reversed-phase column to separate the denatured and un-denatured β -Lg as described previously (Ferreira & Caçote, 2003). The same amount of β -Lg solution, without subjecting to the drying was used as a control. The same pH treatment and centrifugation procedures were followed to this control sample as well. The supernatant of the control samples was also injected in the same way to determine the denatured and un-denatured fractions. A reversed-phase column (Aeris WIDEPOR 3.6 XB-C18, 150×2.1mm, Phenomenex Pty. Ltd., Australia) was used to separate the denatured and un-denatured β -Lg fractions. In these tests a 0.1% (v/v) aqueous solution of TFA was used as solvent A and 0.1% TFA in 80% aqueous acetonitrile (v/v) was used as solvent B. The temperature of the column and the flow rate of the solvent were maintained at 40 °C and 0.5 mL/min, respectively. The injection volume of the sample was 40 μ L. A UV-detector at wavelength of 215 nm was used in these tests. The percentage of denatured β -Lg was calculated using equation (1), as suggested by Parris & Baginski (1991).

$$\text{Denatured protein (\%)} = \left(1 - \frac{A_{\text{treat}}}{A_{\text{control}}}\right) \times 100 \dots\dots\dots (1)$$

where

A_{treat} = area of HPLC peak eluted by dried protein sample

A_{control} = area of HPLC peak eluted by control protein sample

Each of these experiments was carried out in triplicates and the average values are reported.

2.2.3. *Acquiring and analysis the IR spectra*

For structural observation the β -Lg samples were scanned using Perkin Elmer Frontier FT-IR instrument operated by CPU32M software. The sample buffer was prepared by adding

100 mM sodium chloride (NaCl) into deuterium oxide (D₂O) (van de Weert et al., 2001). The dried and control samples were diluted to 5mg/ml β -Lg concentration using the buffer. The SDD samples used in these tests were collected after 600 s of drying at 65 °C and 80 °C. A total of 16 scans at 4 cm⁻¹ resolution were acquired at 0.2 cm/s scanning rate within 650 cm⁻¹ to 4000 cm⁻¹IR region. In order to identify the peak positions, the baseline was subtracted from the acquired spectra to avoid the effect of the buffer. It was followed by a 13-point second derivative analysis to the amide region-I (1600-1700cm⁻¹) by Perkin Elmer's proprietary software (Version 10.03.06).

2.2.4. Modelling of Drying Kinetics using the Reaction Engineering Approach (REA)

Generally, the drying rate of a material can be expressed by equation (2).

$$m_s \frac{dX}{dt} = -h_m A (\rho_{v,s} - \rho_{v,b}) \quad (2)$$

where m_s is the dry solid mass of the sample (kg), X is the average moisture content on dry basis (kg.kg⁻¹), t is the time (s), $\rho_{v,s}$ is the water vapor concentration at interface (kg.m⁻³), $\rho_{v,b}$ is the water vapor concentration in the drying medium (kg.m⁻³), h_m is the mass transfer coefficient (m.s⁻¹) and A is the surface area of the droplet/particle (m²).

The surface vapor concentration ($\rho_{v,s}$) can then be expressed in terms of saturated vapour concentration ($\rho_{v,sat}$) as:

$$\rho_{v,s} = \exp\left(\frac{-\Delta E_v}{RT}\right) \rho_{v,sat}(T) \quad (3)$$

where, ΔE_v (J/mol) represents the additional difficulty to remove moisture from the material when the relative humidity at the surface is less than one. The ΔE_v is moisture content (X)-dependent, R is the universal gas constant (J.mol⁻¹.K⁻¹) and T is the temperature of the

droplet/particle being dried (K). $\rho_{v,sat}$ (kg.m⁻³) is the saturated water vapor concentration which can be estimated as a function of temperature as given by equation (4) (Lin & Chen, 2006, 2007).

$$\rho_{v,sat} = 4.844 \times 10^{-9} (T - 273)^4 - 1.4807 \times 10^{-7} (T - 273)^3 + 2.6572 \times 10^{-5} (T - 273)^2 - 4.8613 \times 10^{-5} (T - 273) + 8.342 \times 10^{-3} \quad (4)$$

The mass balance (equation 2) can be rearranged into equation (5).

$$m_s \frac{dX}{dt} = -h_m A \left[\exp\left(\frac{-\Delta E_v}{RT}\right) \rho_{v,sat}(T) - \rho_{v,b} \right] \quad (5)$$

From equation (5), it can be observed that the REA is expressed as ordinary differential equation with respect to time. It must be noted that the REA does not predict the moisture distribution within the droplet/particle rather it predicts the average moisture content as a function of time.

The activation energy (ΔE_v) can be calculated by rearranging equation (5) into equation (6).

$$\Delta E_v = -RT \ln \left[\frac{-m_s \frac{dX}{dt} \frac{1}{h_m A} + \rho_{v,b}}{\rho_{v,sat}(T)} \right] \quad (6)$$

The rate of moisture loss (dX / dt) is experimentally determined. The dependence of activation energy on average moisture content can be calculated using equation (7).

$$\frac{\Delta E_v}{\Delta E_{v,b}} = f(X - X_b) \quad (7)$$

Where, f is a function of the difference in water content ($X - X_b$), X_b is the equilibrium moisture content under the condition of the drying air (kg.kg⁻¹), $\Delta E_{v,b}$ is the ‘equilibrium’ activation

energy representing the maximum ΔE_v determined by the relative humidity and temperature of the drying, as given by equation (8).

$$\Delta E_{v,b} = -RT_b \ln(RH_b) \quad (8)$$

RH_b is the relative humidity of drying air and T_b is the drying air temperature (K).

In order to predict the temperature profile of the drying droplet, the mass balance equation represented by equation (5) is coupled with the heat balance. For the single droplet drying system mentioned above, the heat balance can be represented by equation (9).

$$mC_{pd} \frac{dT}{dt} = hA(T_b - T) + m_s \frac{dX}{dt} \Delta H_v + 0.5\pi d_f \sqrt{hd_f k_f} (T_b - T) \quad (9)$$

where m is the mass of the droplet/particle (kg), C_{pd} is the specific heat capacity of droplet/particle ($J \cdot kg^{-1} \cdot K^{-1}$), h is the heat transfer coefficient ($W \cdot m^{-2} \cdot K^{-1}$) and ΔH_v is the latent heat of vaporization of water ($J \cdot kg^{-1}$). Similarly, d_f and k_f is the diameter (m) and thermal conductivity $W/(m \cdot K)$ of the glass filament, respectively. The third term in the right-hand side of equation (9) represents the heat conduction through the filament to the droplet. In single droplet drying experiments, the drops were suspended on the tip of a glass filament. Hence, the amount of thermal energy conducted to the droplet through the glass filament should be included in the energy balance equation. The heat conducted through the filament can be quantified and compensated assuming the filament as an infinite fin (Adhikari et al., 2007).

The mass transfer coefficient, h_m and heat transfer coefficient, h can be determined by using the established Ranz-Marshall correlations for Sherwood number (S_h) and Nusselt number (N_u) of spherical droplets subjected to convective air drying (Ranz & Marshall, 1952). In terms of S_h and N_u both the h_m and h can be expressed as given by equations (10) and (11), respectively.

$$S_h = \frac{h_m d}{D_v} = 2 + 0.6 R_e^{0.5} S_c^{0.33} \quad (10)$$

$$Nu = \frac{h d}{k_b} = 2 + 0.6 R_e^{0.5} P_r^{0.33} \quad (11)$$

where, d is the diameter of the evaporating droplet/particle, D_v is the diffusivity of water vapour (m^2/s) and k_b is the thermal conductivity of air ($\text{W.m}^{-1}.\text{K}^{-1}$). R_e , S_c , P_r are dimensionless Reynolds, Schmidt and Prandtl numbers, respectively which were calculated based on the drying air conditions.

The drying conditions of the single droplet drying of the β -Lg droplets are listed in Table 1. The air velocity and the temperature of the drying air were controlled by using precision rotameter (Influx AI29, Influx Measurements Ltd, United Kingdom) and a PID controller (C91 Brain Child electronics, OneTemp Pty Ltd. Australia), respectively. The relative humidity values of the drying air were measured by using EL-USB relative humidity data logger (Lascar Electronics, UK) and were found to be $2 \pm 0.5\%$ at the two sets of drying temperatures. The initial moisture content of droplet was determined based on the mass of the droplet and the solid concentration of the solution while the diameter of the droplet was calculated based on the mass of the droplet and density of the solution and assuming the droplet to be spherical. The physio-chemical properties of β -Lg solution used in the drying kinetics modelling are determined as described in the section below.

2.2.5. Physicochemical properties of β -Lg solution used in the drying kinetics modelling

The equilibrium moisture contents (X_e) of β -Lg were calculated as a function of water activity from sorption isotherm by using Guggenheim–Anderson–de Boer (GAB) model as given by equation (12).

$$X_e = \frac{m_o c k a_w}{(1 - k a_w)(1 - k a_w + c k a_w)} \quad (12)$$

Where, m_o is the monolayer moisture content (kg water/kg dry basis), a_w is the water activity (dimensionless) and c and k are GAB constants.

The moisture adsorption isotherms data for β -Lg were measured at 20 °C and 37 °C and the GAB equation was fitted to these adsorption isotherm data. Method of determination of adsorption isotherms is described in the following section (Section 2.2.6). The GAB model followed the measured adsorption isotherms reasonably well with average absolute error of 4.5±0.5% (Fig. 1). This fitting yielded m_o , c , and k values of 3.37, 2.64 and 0.96, respectively. The data points show that the sorption isotherms at 20 °C and 37 °C are very close to each other. This observation indicates that the effect of temperature on the adsorption isotherm is insignificant compared to the effect of the moisture content in this temperature range (Adhikari et al., 2005). Hence, only the moisture dependence of water activity was considered in the modeling exercise. For REA modelling the equilibrium moisture contents (X_e) of β -Lg were estimated to 0.03 kg water/kg dry solids.

The temperature dependence of density (ρ_p) and specific heat capacity (C_p) of β -Lg was estimated using the Choi and Okos models as given by equations (13) and (14) below (Choi & Okos, 1986).

$$\rho_p = 1329.9 - 0.5184(T - 273.15) \quad (13)$$

where ρ_p is the sample density (kg/m³) and T is the sample temperature (K)

$$C_p = 2008.2 + 1.2089(T - 273.15) - 0.0013129(T - 273.15)^2 \quad (14)$$

where C_p is the sample specific heat (J/kg K)

2.2.6. Adsorption Isotherm measurement

The moisture adsorption isotherms for β -Lg powders were measured using the static gravimetric method (Stitt, 1958; Bronlund & Paterson, 2004). Nine saturated salt solutions were prepared in air tight desiccators with the salts lithium chloride, potassium acetate, magnesium chloride, potassium carbonate, magnesium nitrate, copper chloride, sodium chloride, potassium chloride and potassium sulfate for the corresponding water activities ranging from 0.1 to 0.9. Approximately 5 gm of β -Lg sample from each type were taken in clean and dry pre-weighed petri dishes. The petri dishes were then transferred into the desiccators and incubated at 20 °C and 37 °C. All the samples, in both experimental sets, were allowed to equilibrium over a period of 20 days; until before appearance of any microbe on the samples. The samples were weighed periodically at every alternative day from 14th day of incubation to find equilibrium state of weight. The moisture contents of the samples were determined using oven drying method at 105 °C. Three replications were kept for each sample and the average values were taken as equilibrium moisture contents (EMC) to fit the GAB model.

2.2.7. Modelling of Denaturation Kinetics

The denaturation kinetics of enzymes and proteins during convective drying can generally be expressed by the first-order reaction kinetics model as expressed by equation (15) (Meerdink, G, Van'tRiet, 1995; Haque et al., 2013b).

$$\frac{dC}{dt} = -k_d C \quad (15)$$

where, k_d is the denaturation rate constant (s^{-1}), C is the protein concentration (%) at each time.

277 The denaturation rate constant, k_d can be expressed as a function of temperature and water
278 content of the drying droplet as given by equation (16).

$$279 \quad k_d = k_o \exp\left(aX - \frac{E_a + bX}{RT}\right) \quad (16)$$

280 where, T is the droplet temperature (K), X is the moisture content of the droplet (kg.kg^{-1}), R is
281 the universal gas constant and E_a , is the apparent activation energy (J.mol^{-1}), $k_o(\text{s}^{-1})$, a and b
282 are the model constants.

283 The parameters a , b , k and E_a were estimated considering the simultaneous effects of
284 water concentration (X) and temperature (T) of the drying droplet. A least square algorithm
285 (Microsoft SolverTM, Microsoft Inc., Washington, USA) was used in determining the values of
286 these constants for β -Lg denaturation kinetics data. X and T used in equation (16) are based on
287 the REA-predictions.

288

3. Results and Discussion

3.1. Denaturation of β -Lg during Convective Drying

It can be seen from the elution profile of β -Lg in reversed-phase HPLC (Fig. 2) that the elution times of β -Lg B and β -Lg A are 22.50, and 23.20 min, respectively. This order of elution of these proteins in RP-HPLC is in good agreement with their respective elution time reported in the literature (Parris, N., Baginski, 1991; Haque et al., 2013a). The data presented in this manuscript represent the mean values of the two peaks of β -LgA and β -LgB.

As can be seen from the Fig.3, various drying temperatures caused different degrees of denaturation of β -Lg. When dried at 65 °C for 10 minutes, nearly 24.4% β -Lg was found to be denatured. Higher temperature (80 °C) caused higher degree (30.85%) of denaturation for the same period of drying. The native state of β -Lg was found to decrease significantly when it reached a state of irreversible denaturation (for example at 240s; 80 °C). Before β -Lg reached its state of irreversible denaturation, reversible denaturation occurred. The free cysteine residues present in the β -Lg structure were found to be excited at the state of reversible denaturation. Due to prolonged drying stresses, the peptides of β -Lg were exposed to such an extent that they started to aggregate. However, extent of denaturation of β -Lg in the current study, where the protein was dried individually, is much lower (about 31%) than our previous study (about 70%) during drying of WPI (as a whole) where numerous proteins were present together with β -Lg as mixture (Haque et al., 2013a). The principal reason might be the protein–protein interactions. In aqueous state one group of proteins interact with different proteins as they interact with ions, and other non-protein materials such as lipids, nucleic acids, and carbohydrates (Waugh, 1954). These interactions may range from covalent bonds and electrostatic exchanges to the weaker electronic van der Waals' attractive forces. In a condition likewise the current study where the protein molecule is subjected to a structural change, the

bonding groups of polypeptides bring into positions which allow them to reversible or irreversible structural changes. Such condition can lead to remarkable chemical or physical changes of proteins (Waugh, 1954).

3.2. Effect of drying on the conformation of β -Lg

Amide I region of the infrared spectra was chosen to study the change in the conformational β -Lg during drying. The vibrations in these cases originated mainly from C=O stretching of peptide bonds. These vibrations produced absorption peaks at different location of the IR spectra. However, the original peaks were very intrinsic and could not be separated (Fig. 4A). Therefore, the second derivative mathematical tool was used to locate the secondary structural features (such as α -helix, β -sheet and turn).

Denaturation of protein due to external stress can be identified by observing the shifted, newly appeared and disappeared peaks within the studied region of IR spectra. The peaks from 1620 cm^{-1} to 1640 cm^{-1} , and 1674 cm^{-1} to 1680 cm^{-1} are assigned to the β -sheet secondary structure (Fig. 4B). Similarly, the peaks from 1641-1647 cm^{-1} are assigned to random coil. The peaks appearing in 1648-1660 cm^{-1} are assigned to α -helix, and those appearing in the vicinity of 1663, 1671, 1683, 1694 cm^{-1} are assigned to β -turn (Haque et al., 2015b; Ngarize et al., 2004). It was found that the drying stresses had increased the intensity but narrowed the peaks near 1650 cm^{-1} and 1682 cm^{-1} , and two other peaks appeared at the vicinity of 1618 cm^{-1} and 1662 cm^{-1} . These changes indicate that the proportion of random coil and β -sheet increased due to uncoiling of α -helix, and transformation of β -turn into β -sheet. This type of change in secondary structure is common in many proteins and is attributed to the formation of intermolecular hydrogen-bonded anti-parallel β -sheet structures which leads to aggregation or denaturation (Clark et al., 1981).

3.3. Prediction of Drying Kinetics by REA

For modelling the drying kinetics using the REA, the relative activation energy, which is the fingerprint of the REA, is firstly determined as a function of the moisture content difference ($X-X_b$). Based on the experimental data of moisture content, temperature, and surface area of droplet/particle during drying, the activation energy was calculated from the convective drying run at 80 °C using equation (6). The activation energy was then divided by the equilibrium activation energy (equation 8) to yield the relative activation energy as shown in equation (7). The relative activation energy was then correlated with the moisture content difference ($X-X_b$). The relative activation energy of β -Lg was found to be represented well by equation (17) given below.

$$\frac{\Delta E_v}{\Delta E_{v,b}} = 1 + 826453.696(X - X_b)^{1.806} \exp[-13.666(X - X_b)^{0.181}] \quad (17)$$

As can be seen from Fig.5, equation (17) provides a good fit to the experimental relative activation energy data with $\pm 5.7\%$ errors ($R^2 = 0.946 \pm 0.03$). The relative activation energy was zero at the beginning of drying and keeps increasing during drying. When equilibrium moisture content was attained, the relative activation energy was one as shown Fig. 5. The relative activation energy is essentially material characteristics which represent the difficulty to remove moisture from the materials being dried.

In order to predict the moisture content and temperature profiles during drying, the mass and heat balance equations represented by equations (5) and (9), respectively were simultaneously solved. The equilibrium and relative activation energy, shown in equations (8) and (17), respectively were used for this purpose. The results obtained from the model predictions were then compared with the experimental moisture and droplet temperature history data (shown in Fig. 6 and 7). The results of modelling indicate that the REA-based model accurately describes both moisture content and temperature during drying. The average

errors between the experimental and predicted data for moisture-time and the temperature-time profiles were $\pm 5.80\%$ ($R^2 = 0.935$) and $\pm 3.51\%$ ($R^2 = 0.991$), respectively at drying temperature of 65 °C. Similarly, the average errors between the experimental and predicted moisture content-time and temperature-time profiles were $\pm 5.50\%$ ($R^2 = 0.946$) and $\pm 3.40\%$ ($R^2 = 0.956$), respectively at 80 °C. These results indicate that the REA based temperature and moisture kinetics models can be confidently applied in predicting the moisture and temperature histories of β -Lg droplets in convective drying environment.

3.4. Prediction of Denaturation Kinetics

The experimental and predicted denaturation kinetics of β -Lg at both temperatures (65 °C and 80 °C) are shown in Figs. 6 and 7, respectively. These figures illustrate the effects of dehydration and droplet temperature on the denaturation kinetics. The predicted denaturation kinetics data was generated by using first order reaction kinetics (equations (15) and (16)) in which the REA-predicted moisture content and temperature are incorporated in the model. The model constants are evaluated based on least square method as presented in Table 2. As can be seen from Figs. 6 and 7, the predicted denaturation kinetics data points follow the experimental denaturation kinetics data reasonably well (with $\pm 6.0\%$ errors). The coefficients of determination (R^2) values of these predictions were 0.96 and 0.94 at 65 °C and 80 °C, respectively. The prediction was less accurate in the early stage of drying as reversible denaturation occurred up to 250s (Fig. 3). This reversible denaturation produced fluctuation of hydrophobicity while measuring the denaturation kinetics using RP-HPLC. The activation energies required for the denaturation of β -Lg were found to be 56.28 kJ.mol⁻¹ and 54.72 kJ.mol⁻¹ during drying at 65 °C and 80 °C, respectively. The statistical analysis suggests that the values were not significantly different ($p > 0.05$). The value of the denaturation energy is also comparable with that of inactivation energy of whey protein isolate (WPI) (Haque et al., 2013b).

3.5. Droplet Morphology of β -Lg

The images of β -Lg droplets captured at the beginning and at the end of drying (600 s) are presented in Fig. 8. β -Lg droplet was found to produce buckled particle after drying at both temperatures. Although the β -Lg droplet was not strong enough to resist the deformation of its shape during drying, it had formed firm glassy skin toward the end of the drying.

4. Conclusions

The drying and denaturation kinetics of β -Lg were measured during convective air drying at 65 °C and 80 °C. Both the drying kinetics and denaturation kinetics were predicted using REA-based models, and first order reaction kinetics, respectively. At the end of drying at 65 °C and 80 °C, the denaturation of β -Lg was 24.40% and 30.85%, respectively. The REA-based model predicted moisture and temperature histories of the β -Lg droplets within 5.80% and 3.52% average absolute errors, respectively. Coupling the REA-based model with the first order denaturation kinetics was able to predict the denaturation kinetics of β -Lg both at 65 and 80°C, within 6.20% error. The information generated in this study can be used to tailor the convective drying parameters (especially spray drying ones) to produce minimally denatured β -Lg powders.

REFERENCES

- Adhikari, B., Howes, T., Bhandari, B. R., 2007. Use of solute fixed coordinate system and method of lines for prediction of drying kinetics and surface stickiness of single droplet during convective drying. *Chemical Engineering and Processing*, 46, 405-419.
- Adhikari, B., Howes, T., Lecomte, D., Bhandari, B. R., 2005. A glass transition temperature approach for the prediction of the surface stickiness of a drying droplet during spray drying. *Powder Technology*, 149, 168–179.
- Anandharamakrishnan, C., Rielly, C.D., Stapley, A.G.F., 2007. Effects of process variables on the denaturation of whey proteins during spray drying. *Drying Technology*, 25(5), 799-807.
- Bronlund, J., Paterson, T., 2004. Moisture sorption isotherms for crystalline, amorphous and predominantly crystalline lactose powders. *International Dairy Journal*, 14, 247–254.
- Chatterton, D.E.W., Smithers, G., Roupas, P., Brodkorb, A., 2006. Bioactivity of β -lactoglobulin and α -lactalbumin—Technological implications for processing: A Review. *International Dairy Journal*, 16, 1229–40.
- Chavez, B.E., Ledebor, A.M., 2007. Drying of probiotics: optimization of formulation and process to enhance storage survival. *Drying Technology*, 25 (7-8), 1193–1201.
- Che, L., Li, D., Chen, X.D., 2012. Convective drying kinetics of single droplets of aqueous glucose. *Drying Technology*, 30, 1029–1036.
- Choi, Y., Okos, M.R., 1986. Effects of temperature and composition on the thermal properties of foods. In Food Engineering and Process Applications. Vol. 1, Transport Phenomenon, L. Maguer, P. Jelen (eds), Elsevier; New York, 93–101.

425 Clark, A. H., Saunderson, D.H.P., Suggett, A., 1981. Infrared and laser-Raman spectroscopic
 426 studies of thermally-induced globular protein gels. *International journal of peptide and protein*
 427 *research*. 17, 353-64.

428 Ferreira, I.M.P.L.V.O., Caçote, H., 2003. Detection and quantification of bovine, ovine and
 429 caprine milk percentages in protected denomination of origin cheeses by reversed-phase high-
 430 performance liquid chromatography of beta-lactoglobulins. *Journal of Chromatography. A*,
 431 1015, 111–118.

432 Haque, M. A. Aldred, P., Chen, J., Adhikari, B., 2015a. Drying and denaturation characteristics
 433 of whey protein isolate in the presence of lactose and trehalose. *Food Chemistry*, 177, 8-16.

434 Haque, M. A., Putranto, A., Adhikari, B., 2016. Predictions of Drying Kinetics of Aqueous
 435 Droplets Containing WPI-Lactose and WPI-Trehalose by Application of Composite Reaction
 436 Engineering Approach (REA). *Journal of Food Engineering*, 189, 29-36.

437 Haque, M.A., Aldred. P., Chen, J., Barrow, C.J., Adhikari, B., 2013a. Comparative study of
 438 denaturation of whey protein isolate (WPI) in convective air drying and isothermal heat
 439 treatment processes. *Food Chemistry*, 141, 702-711.

440 Haque, M. A., Putranto, A., Aldred. P., Chen, J., Adhikari, B., 2013b. Drying and denaturation
 441 kinetics of whey protein isolate (WPI) during convective air drying process. *Drying*
 442 *Technology*, 31 (13-14), 1532-1544.

443 Haque, M.A., Adhikari, B., 2014. Drying and denaturation of proteins in spray drying process.
 444 In A. S. Mujumdar (Ed.), *Handbook of industrial drying*, 4th Ed. *CRC Press, Taylor and*
 445 *Francis group, NW, USA*, pp 971-984.

446 Haque, M.A., Chen, J., Aldred, P., Adhikari, B., 2015b. Denaturation and Physical
 447 Characteristics of Spray Dried Whey Protein Isolate Powders Produced in the Presence and
 448 Absence of Lactose, Trehalose and Polysorbate 80. *Drying Technology*, 33, 1243-54.

449 Ikeuchi, T., Aoki, T., Yoshida, T., Takahashi, K., Hattori, M., 2008. Functional Improvements
 450 to β -Lactoglobulin by Preparing an Edible Conjugate with Cationic Saccharide Using
 451 Microbial Transglutaminase (MTGase). *Bioscience, Biotechnology and Biochemistry*, 72(5),
 452 1227–34.

453 Jimenez-Castano, L., Villamiel, M., Martín-Alvarez, P.J.,
 454 Olano, A., López-Fandinó, R., 2005. Effect of the dry-heating conditions on the
 455 glycosylation of β -lactoglobulin with dextran through the Maillard reaction. *Food*
 456 *Hydrocolloids*, 19, 831–37.

457 Lin, S. X. Q., Chen, X. D., 2007. The reaction engineering approach to modelling the cream
 458 and whey protein concentrate droplet drying. *Chemical Engineering and Processing*, 46, 437-
 459 443.

460 Lin, S.X.Q., Chen, X. D., 2006. A Model for Drying of an Aqueous Lactose Droplet Using the
 461 Reaction Engineering Approach. *Drying Technology*, 24, 1329–1334.

462 Meerdink, G, Van'tRiet, K., 1995. Prediction of product quality during spray drying.
 463 *Transactions of the IChemE Part C: Food and Bioproduct Processing*, 73, 165-170.

464 Ngarize, S., Herman, H., Adams, A., Howell, N., 2004. Comparison of changes in the
 465 secondary structure of unheated, heated, and high-pressure-treated β -lactoglobulin and
 466 ovalbumin proteins using Fourier transform Raman spectroscopy and self-deconvolution.
 467 *Journal of Agricultural and Food Chemistry*, 52, 6470-6477.

468 Parris, N., Baginski, M. A., 1991. A rapid method for the determination of whey protein
 469 denaturation. *Journal of Dairy Science*, 74, 58-64.

470 Patel, K. C., Chen, X. D., 2005. Prediction of spray-dried product quality using two simple
 471 drying kinetics models. *Journal of Food Process Engineering*, 28, 567–594.

472 Ranz, W. E., Marshall, W. R., 1952. Evaporation from drops. *Chemical Engineering and*
 473 *process*, 48(3), 141-146.

474 Sano, Y., Keey, R.B., 1982. The drying of a spherical particle containing colloidal material
 475 into a hollow sphere. *Chemical Engineering Science*, 37(6), 881-889.

476 Shpigelman, A., Israeli, G., Livney, Y.D., 2010. Thermally-induced protein polyphenol co-
 477 assemblies: beta lactoglobulin-based nanocomplexes as protective nanovehicles for EGCG.
 478 *Food Hydrocolloids*, 24, 735-43

479 Stitt, F., 1958. Moisture equilibrium and the determination of water content in dehydrated
 480 foods. In Society of Chemical Industry. Fundamental aspects of the dehydration of foodstuffs,
 481 Metchim and Son, London. pp. 67–87.

482 van de Weert, M., Haris, P.I., Hennink, W. E., Crommelin, D.J.A., 2001. Fourier Transform
 483 Infrared Spectrometric Analysis of Protein Conformation: Effect of Sampling Method and
 484 Stress Factors. *Analytical Biochemistry*, 297,160-169.

485 Waugh, D. F. 1954. Protein-Protein Interactions. *Advances in Protein Chemistry*, Volume
 486 9, 325-437.

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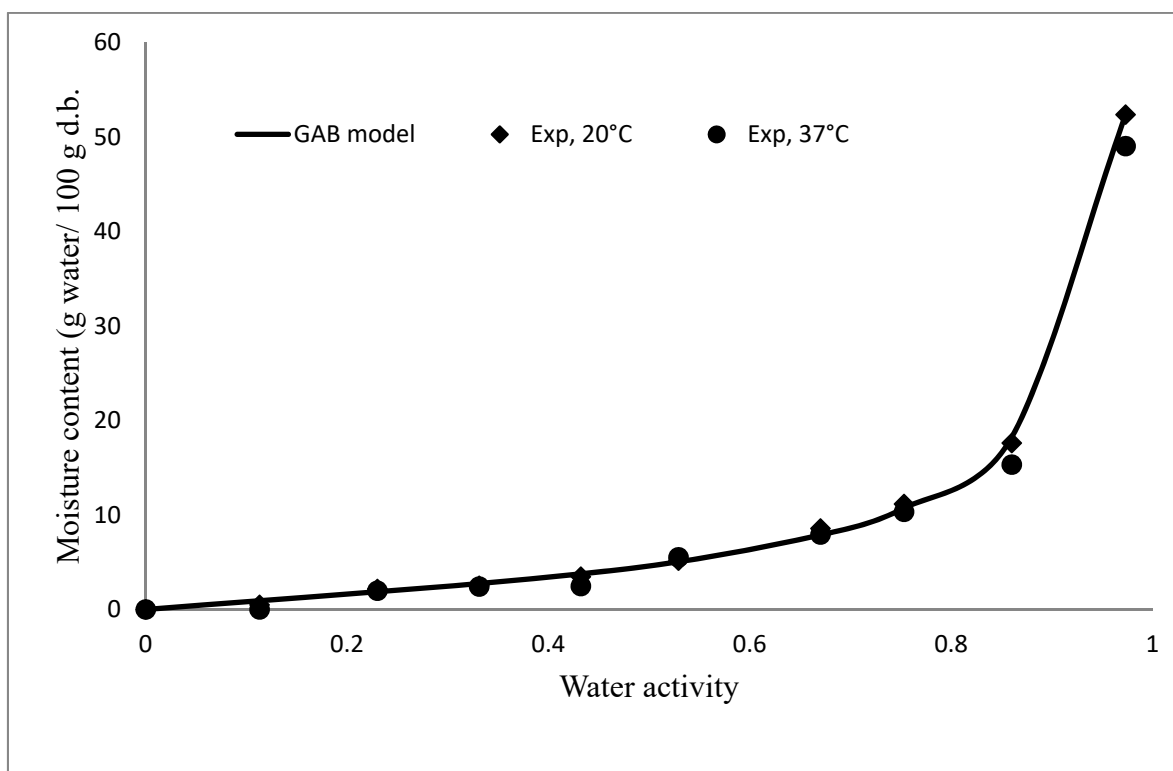
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Table 2. Kinetic parameters in prediction of denaturation of β -Lg during droplet drying

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FIGURES



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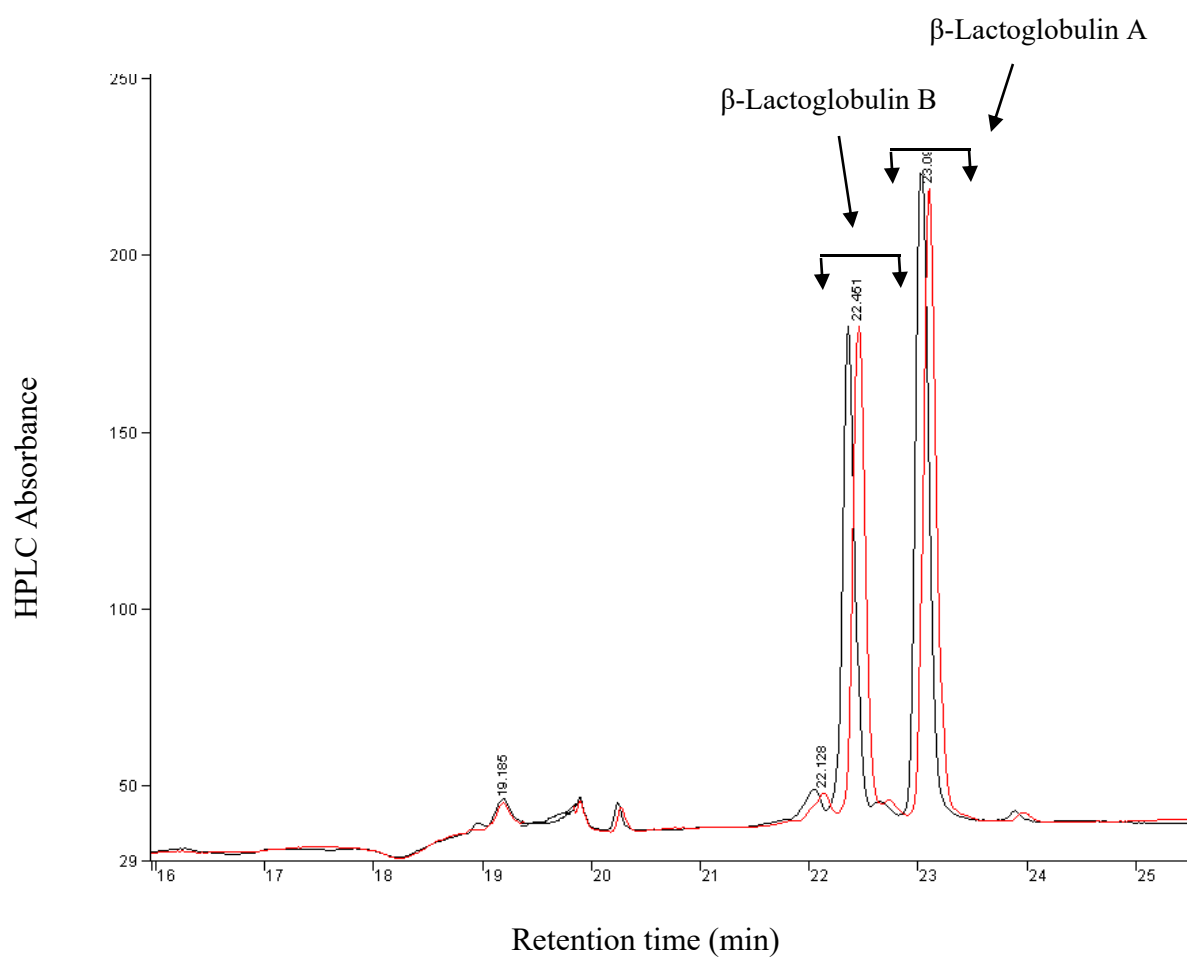


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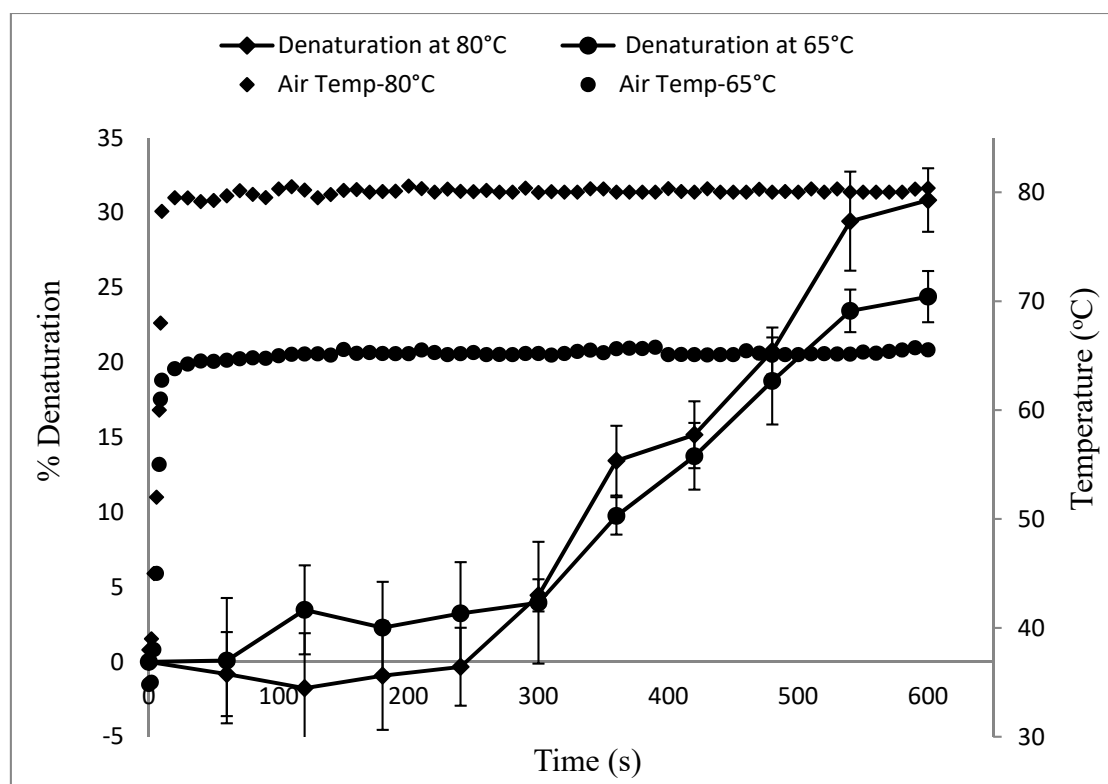
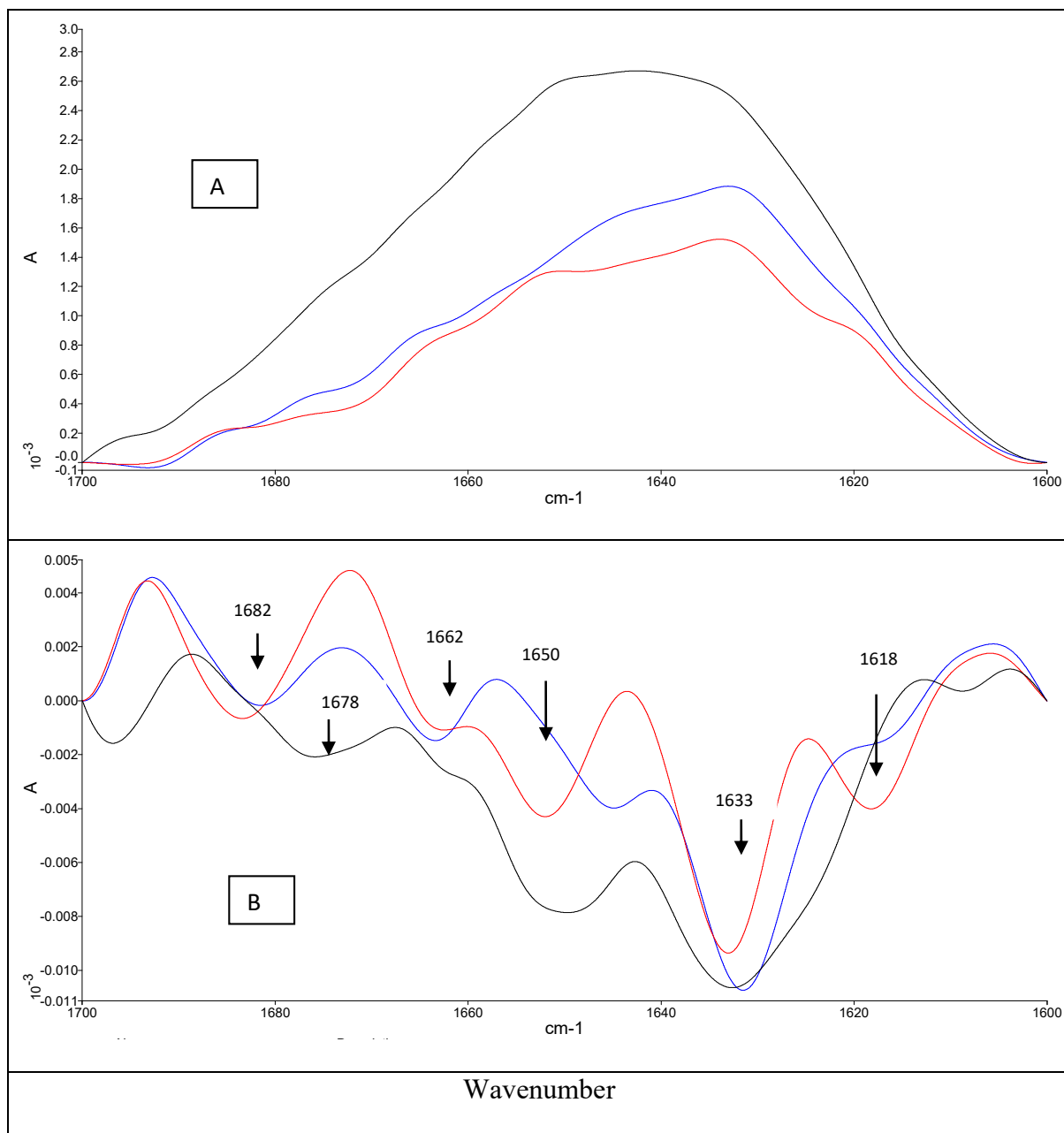


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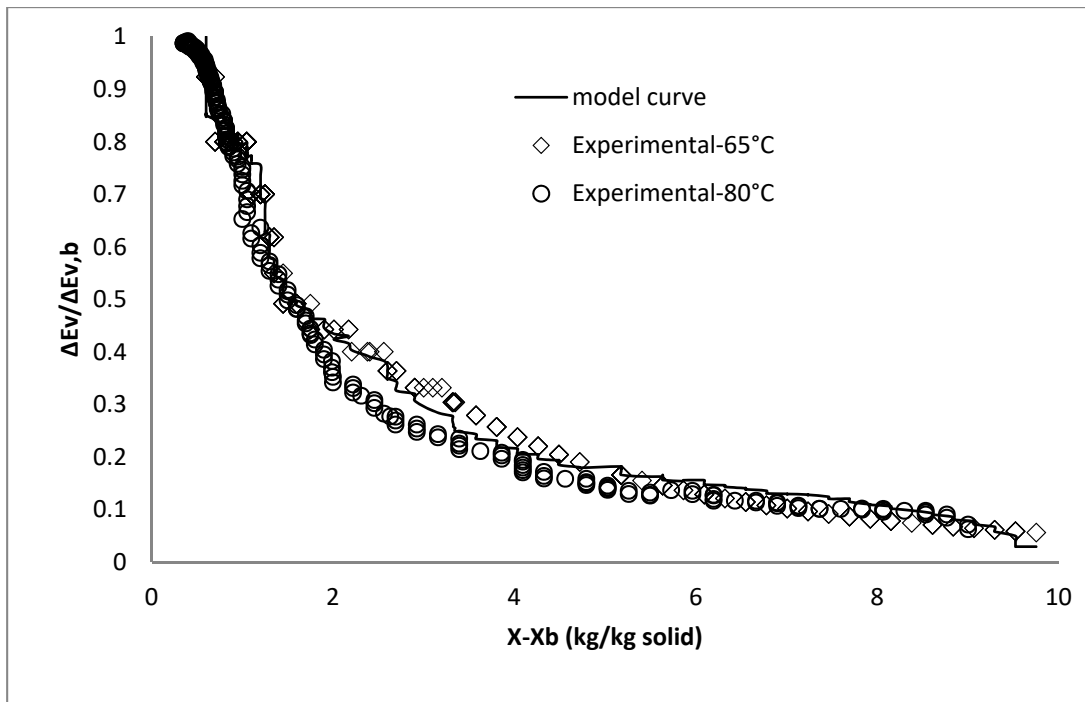
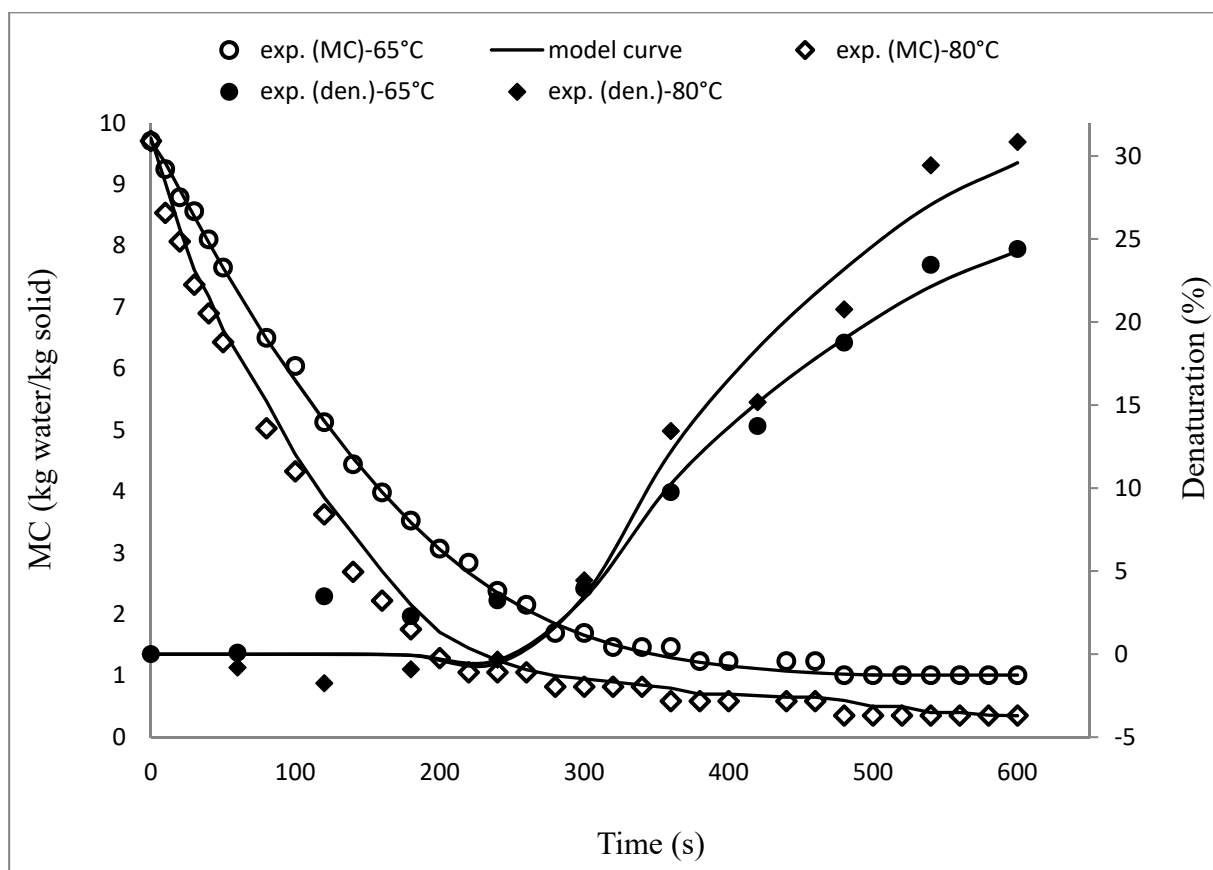


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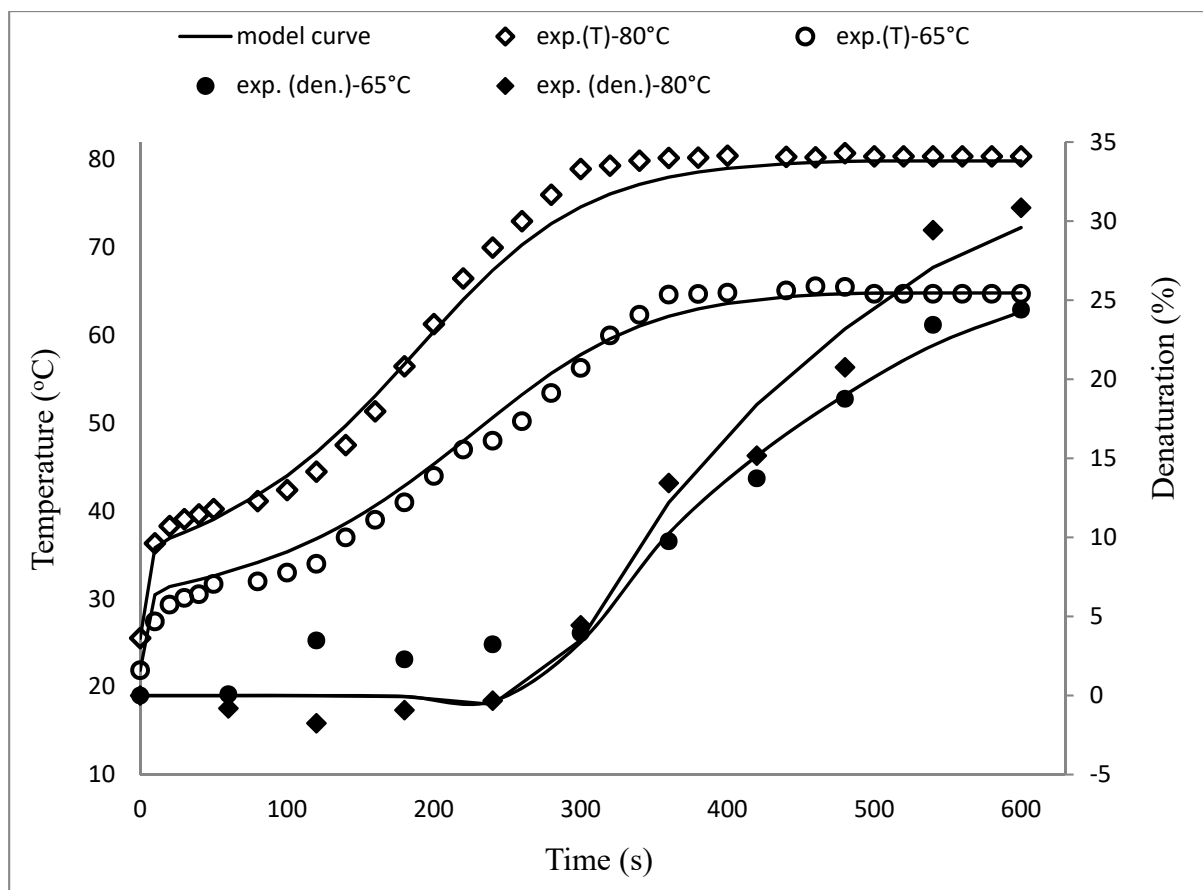
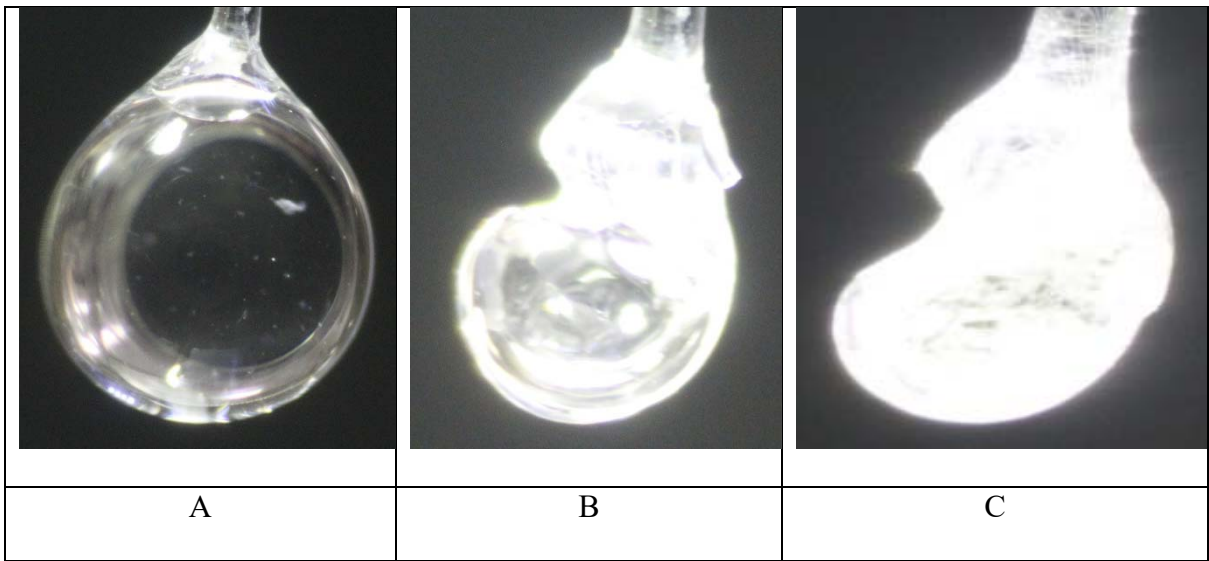


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TABLES

564 Table 1

565 The drying conditions during single droplet drying of β -Lg

Experimental conditions	65 °C drying air	80 °C drying air
Initial temperature (°C)	23.65	24.56
Initial moisture content (kg/kg dry solid)	9.75	9.75
Drying air velocity (m/s)	0.5	0.5
Relative humidity (%)	2.5±0.5	2.5±0.5
Initial droplet diameter (m)	0.002	0.002

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567 Table 2

568 Kinetic parameters in prediction of denaturation of β -Lg during droplet drying

Drying temperature	$K_o(s^{-1})$	a	b	E_a (kJ.mol ⁻¹)	R^2	% error
65 °C	80867	306	400743	56.28	0.960	5.95
80 °C	79005	298	354023	54.72	0.935	6.01

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