STATE DIAGRAM AND WATER ADSORPTION ISOTHERM OF RASPBERRY (*Rubus idaeus*)

Roopesh M. Syamaladevi[1], Shyam S. Sablani[1]*, Juming Tang[1],
Joseph Powers[2] and Barry G. Swanson[2]

[1]*Biological Systems Engineering Department, Washington State University, P.O Box 646120, Pullman WA 99164-6120, USA*

[2]*Department of Food Science and Human Nutrition, Washington State University, P.O Box 6463760, Pullman WA 99164-6376, USA*

***Corresponding author**
(Email: ssablani@wsu.edu; Tel: +509 335 7745; Fax: +509 335 2722)

ABSTRACT

Thermal transitions of freeze-dried raspberry powder (*Rubus idaeus*) were analyzed by using differential scanning calorimetry. Freeze-dried raspberry powders containing unfreezable and freezable water were examined to develop a state diagram of raspberry. The state diagram of freeze-dried raspberry powders included the glass line; glass transition temperature *versus* solids content, freezing curve; initial freezing point *versus* solids content; end point of freezing T_m', corresponding solids content X_s', characteristic glass transition T_g' and corresponding solids contents X_s'' of maximally-freeze-concentrated raspberry. The conditions of the maximal-freeze-concentrate obtained from freezing curve corresponded to T_m' = -38°C and X_s' =0.78 kg solids/kg raspberry and T_g' = -47°C and X_s''= 0.82 kg solids/kg raspberry. The T_g' was determined by extending the freezing curve to glass line. The quantities of unfreezable water identified from enthalpy data and the freezing curve were comparable. Adsorption isotherms of freeze-dried raspberries were determined at room temperature by the isopiestic method and the data was modeled with BET and GAB equations. The BET and GAB monolayer moisture contents were observed as 0.045 and 0.074 kg water/kg dry raspberry solids, respectively. The state diagram and water sorption properties of raspberries are useful in optimizing the retention of anthocyanins, phenolics concentrations and antioxidant activities in freeze-dried and frozen raspberries during storage.

***Keywords*:** Differential scanning calorimeter, glass transition, Gordon-Taylor equation, maximal-freeze-concentration, state transition, water activity, GAB model

INTRODUCTION

A state diagram of food presents different physical states of food as a function of solids content and temperature. The role of the state diagram of food materials in determining processing and storage stability is highlighted in a number of studies (Rahman 2006; Sablani et al., 2004; Champion et al., 2000; Goff and Sahagian, 1996; Sa and Sereno, 1994; Roos and Karel, 1991; Slade and Levine, 1991). The state diagram consists of a freezing curve of initial freezing point *versus* solids content, a solubility curve of solids fraction in a saturated aqueous solution at a given temperature, the eutectic point, a glass line of glass transition temperature *versus* solids content, and conditions of maximal-freeze-concentration (Rahman, 2006). The concept of glass transition was investigated extensively in polymer, material, pharmaceutical and food sciences to relate physical, chemical and structural changes in the physical state of material. Glass transition is a nature of second order time-temperature dependent transition of physical state of a material. During glass transition temperature change, material transforms from a relatively stable glassy state to a metastable rubbery state or *vice versa*. As a result of the industrial relevance and scientific interest of glass transition research, researchers continue to discuss the application of glass transition as a tool for predicting the microbiological, physical and chemical changes that occur during processing and storage (Sablani et al., 2007a-c; Kasapis et al., 2007; Rahman, 2006; Khalloufi and Ratti, 2003; Champion et al., 2000; Karel et al., 1994; Kerr et al., 1993; Roos and Karel, 1991; Slade and Levine, 1991).

Raspberries (*Rubus idaeus*) are commercial fruits used industrially for formulating jam, jelly, sauce, puree, topping, syrup or juice concentrates. Raspberry fruit is well recognized for health promoting constituents. Raspberries are rich in potential antioxidant phenolic compounds including anthocyanins. Studies evaluate the potential role of raspberries in preventing chronic stress, cancer and heart diseases (Zhang et al., 2005; Wang et al., 2000). Anthocyanins and phenolic compounds are susceptible to deterioration during processing and storage conditions (Sadilova et al., 2006). Stability of bioactive compounds during processing and storage is important to the food industry.

Glass transition temperature data are reported for several fruits (tomato, dates, pineapple and grapes) but a complete state diagram using glass lines and freezing curves are reported only for selected fruits (apples, strawberries, grapes and dates) (Bai et al., 2001; Kasapis et al., 2000; Rahman, 2004; Sa and Sereno, 1994; Sa et al., 1999). Khalloufi et al. (2000) examined glass transition temperatures of raspberries, blueberries, strawberries and blackberries as a function of water contents. The glass transition temperatures of the berries decrease as water contents increase. Since soluble solids of berries are mostly sugars, the glass transition temperature of the freeze-dried powder is associated with the glass transition temperatures of glucose and fructose. However the studies related to freezing curve and conditions of maximally freeze-concentration for berries including raspberries are not reported in the literature. This information on maximal freeze concentration of berries is important to develop a complete state diagram useful in studying stability of anthocyanins and other bioactive compounds in frozen and dried raspberries.

The objectives of the current study is to develop a state diagram for freeze-dried raspberries by determining glass line (T_g *versus* total solids content), freezing curve (initial freezing temperature *versus* total solids content) and maximal-freeze-concentration conditions (T_m', T_g', X_s'). In addition, a water adsorption isotherm is determined to evaluate and compare a stability criterion with the concept of glass transition.

MATERIALS AND METHODS

Red raspberry fruits (*Rubus idaeus*) grown in Vancouver, WA were collected and frozen immediately at -37°C for 48 h. The frozen raspberries were layered in the metal trays of freeze dryer (Virtis freeze mobile 24 with Unitop 600L, VirTis SP Industries Co., New York) to decrease the water content. The shelf temperature was set at -20°C with a vacuum of 20 Pa. The temperature of the condenser was adjusted to -60°C. After 48 h of freeze drying, the raspberries were removed and ground immediately to a fine powder with a mortar and pestle. The moisture content of the raspberry powder was 0.042 kg H_2O/kg dry raspberry solids. The raspberry powder was placed in open weighing bottles and equilibrated for three to four weeks with saturated salt solutions of constant water activities in airtight containers (volume: 2.5×10^{-3} m^3) at room temperature (23°C). The salts used were: LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, $MgNO_3$, $NaNo_2$, NaCl and KCl (Fisher Scientific, Houston, TX) with equilibrium relative humidities of 11.3, 22.5, 32.8, 43.2, 52.9, 65.8, 75 and 86%, respectively. Relative humidity values for the saturated salt solutions were obtained from Greenspan (1977). A small amount of thymol was placed inside the airtight containers to avoid microbial growth in raspberry powders.

After equilibration triplicate of 1 g raspberry powder samples were used to determine the water content in a vacuum oven. For this, raspberry powders in aluminum weighing dishes were placed inside a vacuum oven at 80°C for 10 h. The pressure inside the chamber was 10 kPa. The dried raspberry powders obtained after vacuum oven drying were stored under dark and dry conditions for thermal transition experiments. Triplicate samples of high moisture raspberry powders (0.30, 0.40, 0.50, 0.60, 0.70 and 0.80 kg H_2O/kg raspberry) were prepared by adding precalculated amount of distilled water to the dried raspberry powders obtained after the freeze drying. The raspberry powders were mixed with water in a small beaker and sealed with aluminum foil to avoid moisture loss. The prepared raspberries were equilibrated at 4°C for 24 h before experimentation.

Determination of thermal transitions

The thermal transition experiments in freeze-dried raspberry powder were conducted with a differential scanning calorimeter (DSC, Q2000, TA Instruments, New Castle, DE). The calorimeter was calibrated by checking standard temperatures and enthalpies of fusion for indium and sapphire. The raspberry powders were cooled by a mechanical refrigerated cooling system. An empty sealed aluminum pan was used as a reference in each test. Following equilibration, 10-20 mg raspberry powders were sealed in aluminum pans (volume 30μL) and cooled from room temperature to -90°C at 5°C/min and equilibrated for 10 min. Raspberry powders were scanned from -90°C to 70°C at a rate of 5°C/min. Initially selected sample with moisture content of 0.034 kg H_2O/kg raspberry powder was scanned at 1, 2, 5, 10 and 20°C/min and scan rate of 5°C/min was selected for subsequent analysis. The scan rate of 5°C/min is commonly used for determination of glass transition temperature. DSC produces heat flow (W/g) versus temperature thermograms. The glass transition temperature (T_g) is identified as a (vertical) shift in the heat flow curve of thermogram. TA Instruments Universal analysis software was used to analyze the onset, mid and end points of the glass transition. Three replicates were used for the determination of glass transition temperatures at each water content/water activity. In addition, freeze-dried raspberry powders with moisture of 0.042 kg H_2O/kg raspberry were further dried in a vacuum oven to obtained raspberry powder with no moisture for thermal analysis. For high moisture raspberry powders, thermograms provide melting endotherms along with glass transition temperatures. The area of the melting endotherm peaks provides the enthalpy of melting (ΔH_m) determined by drawing a linear base line to the endotherm. The intersection point of the baseline with the left side of the endotherm was taken as the end point of freezing (T_m') of the raspberries. High moisture raspberry powders (0.3–0.8 kg H_2O/kg raspberry) were subjected to annealing at a temperature (T_m'-1) for 30 min during a DSC scan. Initially annealing was performed on raspberries with moisture content of 0.4 kg H_2O/kg raspberry at a temperature (T_m'-1) for 0, 30 and 60 min and an annealing time of 30 min was chosen for further analysis. After annealing, freeze-dried raspberry powders were scanned from (T_m'-1) to -90°C at the rate of 5°C/min. From -90 °C to 70°C, raspberries were scanned at a rate of 5°C/min. A tangent to the left side of the endotherm curve was drawn to identify the freezing point (T_F) of the high moisture raspberries (Rahman, 2004; Bai et al., 2001).

Water sorption and thermal transitions modeling

Several theoretical (BET, GAB model etc) and empirical equations (Oswin, Henderson model etc.) are available for modelling of sorption isotherms data. In the present study water adsorption data of freeze dried raspberry powder was modeled using most commonly used Brunauer-Emmett-Teller (BET) and Guggenheim-Andersen-de

Boer (GAB) equations (Rahman, 1995). Both of these models have sound theoretical background and their parameters provide physical meaning related to the sorption process compared to the empirical models (Labuza and Altunakar, 2007). These two models are based on the monolayer moisture concept and provide the value of the monolayer moisture content of the material, considered the safe moisture for dried foods during preservation, but most other models lack this parameter. The BET equation is:

$$M_w = \frac{M_b B a_w}{(1-a_w)[1+(B-1)a_w]} \tag{1}$$

Where M_w is the water content (kg water/kg dry solids), M_b is the BET monolayer water content (kg water/kg dry solids) and B is a constant related to the net heat of sorption. The value of B is normally less than 2 for type III isotherms and varies between 2 and 50 for type II isotherm. The BET isotherm is applicable between water activities of 0.05 and 0.45, an adequate range for the calculation of parameters M_b and B (Rahman, 1995). The GAB equation is:

$$M_w = \frac{M_g C K a_w}{[(1-Ka_w)(1-Ka_w+CKa_w)]} \tag{2}$$

where M_g is the GAB monolayer water content (dry basis). C is a constant related to the monolayer heat of sorption and the value of C varies from 1 to 20. K is a factor related to the heat of sorption of the multilayer and the value of K varies from 0.7 to 1. BET and GAB models are the most commonly used models to fit sorption data of food materials. The GAB isotherm equation is an extension of the BET model taking into account the modified properties of the sorbate in the multilayer region and the bulk liquid water properties through the introduction of a third constant K. Estimation of three parameters in GAB using water content and water activity variables leads to non-linear optimization.

The BET monolayer value is more acceptable than GAB monolayer value, although the GAB model provides accurate prediction for water activities range less than 0.90 (Rahman, 1995).

The glass transition temperature of amorphous foods is influenced by water content. The influence of water content on glass transition temperature is commonly modeled by the Gordon and Taylor (1952) equation:

$$T_{gm} = \frac{X_s T_{gs} + kX_w T_{gw}}{X_s + kX_w} \qquad (3)$$

where T_{gm}, T_{gs} and T_{gw} are the glass transition temperatures of the mixture, solids and water, respectively; X_w and X_s are the mass fraction of water and total solids (wet basis), and k the Gordon-Taylor parameter. From the thermodynamic standpoint the k parameter is equivalent to the ratio of the change in specific heats of the components of the mixture at their T_g. The model parameters (k and T_{gs}) of Equation (3) were estimated using non-linear regression analysis while considering $T_{gw} =$ -135°C.

The Clausius-Clapeyron equation was used to model the freezing line of dried raspberry powder with change in water content. The Clausius-Clapeyron equation is expressed as (Rahman, 1995; Sablani et al., 2004):

$$\delta = -\frac{\beta}{\lambda_w} \ln\left[\frac{1 - X_s}{1 - X_s + EX_s}\right] \qquad (4)$$

In equation (4), δ is the freezing point depression (T_w - T_F) relative to increasing total solid contents, T_F is the freezing point of the food material (°C), T_w is the freezing point of water (°C), β is the molar freezing point constant of water (1860 kgK/kg mol), λ_w is the molecular mass of water, X_s is the solids mass fraction and E is the molecular mass ratio of water to solids (λ_w/λ_s).

Use of Clausius-Clapeyron equation is limited to ideal and dilute solutions. The Chen model is an extension of Clausius-Clapeyron equation by the introduction of a new parameter B, which is the ratio of unfreezable water to the total solids content. The equation (4) is expressed as (Chen, 1986):

$$\delta = -\frac{\beta}{\lambda_w}\ln\left[\frac{1 - X_s - BX_s}{1 - X_s - BX_s + EX_s}\right] \tag{5}$$

The parameters E and B were estimated using non-linear optimization analysis by EXCEL© solver. In the present study, the Chen model was selected to fit the freezing point data to the experimental data.

RESULTS AND DISCUSSION

Adsorption isotherm of freeze-dried raspberries

The water adsorption isotherm of freeze-dried raspberry powders at 23°C followed a typical type II behavior as presented in Figure 1. The adsorption isotherm of freeze-dried raspberry powders exhibits a sigmoid shape with three distinct regions a_w = 0.0 to 0.25, 0.25 to 0.6 and 0.6 to 0.8 typical to type II isotherm. The sigmoid shape of sorption isotherm is common for many food and biological materials (Rahman, 1995; Rahman and Labuza, 1999).

The sorption isotherm data was modeled using BET and GAB models. The model constants in BET model were M_b = 0.059 kg H_2O/kg dry raspberry solids, B = 9.08 and R^2 = 0.968; whereas in GAB model the constants were M_g = 0.074 kg H_2O/kg dry raspberry solids, C = 5.53 and K = 0.904. The model constants in the BET and GAB are temperature dependent (Rahman, 1995; Lim et al., 1995). However in the present study water sorption experiments were performed at room temperature (~23°C). The *C* values vary from 1 and 20 and the *K* values vary from 0.7 to 1 for many food materials (Rahman, 1995). However in some instances the *K* values are reported greater than 1, such as for freeze-dried raspberries (=1.02) (Khalloufi et al., 2000). *K* values > 1 are mainly due to the nonlinear optimization procedure used to determine three parameters in the GAB model using only water content and water activity as inputs. The GAB monolayer moisture content value of the freeze-dried raspberry powders obtained in the current study is smaller than the GAB monolayer moisture content reported by Khalloufi et al. (2000) (Table 1). This difference might be due to the difference in cultivars of raspberries and the chemical composition which may result in deviation in the values of GAB constants. Khalloufi et al. (2000) used five equilibrium relative humidities to obtain equilibrium moisture content, while eight different relative humidities were used in the present study to estimate equilibrium moisture contents which may have caused some difference in GAB constants.

Thermal transitions of freeze-dried raspberries containing unfreezable water

The thermograms of freeze-dried raspberry powders obtained in a single scan corresponding to water activities in the range of 0.11 to 0.86 are presented in Figure 2. This figure presents only portion of thermograms around the glass transition temperature for raspberry powders of different water activities. Within water activities range of 0.11 to 0.86, thermograms exhibited only one transition and no formation of ice and no ice melting endotherm. This nature of the thermograms was similar to thermograms in the literature for selected fruits (Khalloufi et al., 2000; Roos, 1987; Telis and Sobral, 2001 and 2002; Sa and Sereno, 1994). The glass transition temperatures of foods depend mainly on the quantity of water, constituents and molecular weight of solutes present in the food. The glass transitions temperatures in foods are not sharp but occur over a range of temperature (Rahman, 2006). The initial (T_{gi}), mid (T_{gm}), and end-points (T_{ge}) of the glass transitions were determined from thermograms (Table 2). The DSC scan rates during experiment to influence glass transition temperatures. Higher scan rates introduce thermal lag between heating element and the sample resulting in non uniform temperature distribution in the sample (Tang et al., 1991). The glass transitions occurred at lower temperature with decreasing rates of cooling (scan rates) (Figure 3). The scan rates of 5°C/min were considered as optimal rates of scanning since at lower scan rates change in transition temperatures was minimal. In the literature, scan rates of 5°C/min are most commonly used for thermal transition experiments to pinpoint glass transition temperatures of foods (Rahman, 2004). The glass transition temperatures of freeze-dried raspberry powders are influenced by water content. The T_{gi} decreased from 17.5°C to -65.5°C as water content of the freeze-dried raspberries increased from 0.034 to 0.242 kg water/kg raspberry powder. The depression in glass transition temperatures with increasing water content is due to the plasticization effect of water on the amorphous constituents of the matrix. Fresh raspberries contain 84.5% water, 13.4% carbohydrate, 1.30% protein, 0.3 % fat and 0.5% ash (Khalloufi et al., 2000). Glucose and fructose are the major sugars present in raspberries. The glass transition temperatures in raspberries are related to the T_g of glucose and fructose. The glass transition temperatures and thermograms of freeze-dried raspberries are similar to the glass transition temperatures and thermograms of glucose and fructose (Ablett et al., 1993; Simperler et al., 2006; Roos, 1993).

Thermal transitions of raspberries containing freezable water

For raspberries containing freezable water (0.30 to 0.70 kg water/kg raspberry), glass transition temperatures were less noticeable before ice melting. The raspberry powders were first scanned without annealing to identify the end point of freezing or start of melting of ice crystals T_m'. The freeze-dried raspberry powders were rescanned with annealing for 0 to 60 min at T_m'-1. The optimal annealing conditions are obtained when the raspberry powder is held for a considerable period of time, allowing the formation of maximum amount of ice and leading to a maximally freeze-concentrated solid matrix. However, for raspberry powders with large water content (a_w>0.90) a shorter period of annealing is sufficient for maximal ice formation due to the large amount of freezable water (Sa and Sereno, 1994; Bai et al., 2001). The effect of annealing time was analyzed for raspberries containing freezable water (0.4 kg water/kg raspberry) (Table 3). As expected, glass transition temperature decreased and clear discontinuities in thermograms were observed as annealing time increased from 0 to 60 min. The decrease in glass transition temperatures is related to molecular relaxation occurring inside the materials analyzed during annealing (Rahman, 2004). An annealing time of 30 min was taken as optimal for further analysis.

The initial freezing points (T_F), end point of freezing (T_m'), and enthalpy of ice melting (ΔH_m) of freeze-dried raspberries were determined from thermograms obtained for high water content raspberries (Figure 4). The T_F decreased from -2.45 to -17.4°C as total solids content (X_s) increased from 0.3 to 0.7 kg solids/kg raspberry (Table 4). The magnitude of freezing point temperature depression due to increasing total solids depends on the molecular weight of the solids. The area of the melting peak provided enthalpy of ice melting in the raspberries, which decreased as solids content increased from 0.3 to 0.7 kg solid/kg sample. The enthalpy of ice melting was plotted against water content to determine the quantity of unfreezable water. A linear relationship was obtained between enthalpy and water content data for freeze-dried raspberries. The amount of unfrozen water was 0.16 kg water/kg raspberry by extending the line to ΔH_m equal to zero (Figure 5). The quantity of unfreezable water observed for grapes, strawberries, garlic, date flesh, and pineapple were 0.197, 0.184, 0.20, 0.32, and 0.30 kg water/kg sample respectively (Sa and Sereno., 1994; Rahman et al., 2005; Rahman., 2004; Telis and Sobral., 2001). Bound water is the water with great affinity attached to solute molecules in foods. Bound water may be unavailable for chemical reactions and may not be freezable at low temperatures. While all unfreezable water may not be bound to the solute molecules (Franks, 1986). Bound water may be considered as a fraction of unfreezable water. The amount of bound and unfreezable water depends on the molecular weight of solutes present in foods.

Similar to equilibrium freezing point, the T_m' is also influenced by the molecular weight of total solids present in foods. However the determination of T_m' becomes complex and in some cases varies with total solids content, which may be a result of the kinetics of the system (Rahman et al., 2005). The T_m' decreased with increasing solids content; however, at solids content greater than 40% the change in value of T_m' is small (Sopade et al., 2002; Rahman, 2004; Rahman et al., 2005). The T_m' of raspberry was -

38.0°C at a total solids content between 0.50 and 0.70 kg solids/kg raspberry. The T_m' of raspberry is in the range of sugars i.e. T_m' of glucose and fructose (T_m' = -30°C to -48°C) (Roos, 1992).

State diagram

The stability and shelf-life of low moisture and frozen foods can be evaluated using state diagrams (Roos and Karel, 1991; Rahman, 2006). The state diagram defines the parameters of physical state as well as state transitions of biomaterials. Experimentally determined glass transition temperatures, equilibrium freezing points and the conditions of maximal-freeze-concentration were used to construct the state diagram of raspberries (Figure 6). The freezing curve *AM* (equilibrium freezing point versus solids content) was modeled using Chen's equation (5). The parameters in the Chen equation *E* and *B* were estimated using nonlinear optimization technique as 0.064 and 0.141, respectively. The *E* values reported for apples, dates and garlic are 0.238, 0.129 and 0.080 respectively (Bai et al., 2001; Rahman 2004; Rahman et al., 2005). The *B* value is the unfrozen water per unit dry solids (kg water/kg dry solids) and a *B* value of 0.141 (dry basis) corresponds to unfreezable water of 0.124 kg water/kg raspberry (wet basis). The value of unfreezable water from Chen model is comparable to the unfreezable water from enthalpy data. The value of X_s' (total solids content corresponding to T_m') was determined by extending the freezing curve to -38°C (point *M*) and corresponding total solids was estimated by trial and error using equation (5). The values of X_s' and unfreezable water content corresponding to T_m' were 0.78 kg solids/kg raspberry and 0.22 kg H_2O/kg raspberry, respectively.

Water acts as a plasticizer in a multicomponent system containing solutes. The effect of water concentration on glass transition is predicted by the Gordon-Taylor equation (Gordon and Taylor, 1952) based on ideal volume mixing. The glass line (*DE*) is depicted by fitting the Gordon-Taylor (GT) equation with the experimental T_{gi} *versus* solids content. The GT constants T_{gs} and k were determined as 42.6°C and 4.73, respectively by a non-linear optimization technique. The GT constants obtained for freeze-dried raspberries were in the range reported for other berries and the prominent sugars (glucose and fructose) present in raspberries (Table 1). The experimental values of T_{gi}, T_{gm} and T_{ge} obtained for anhydrous raspberry powder were 37.3, 40.2 and 42.0°C, respectively. The experimental T_{gi} value of 37.3°C of dry raspberry powder was lower than the value of 42.6°C predicted by the GT equation. To identify the glass transition temperature corresponding to the maximally freeze concentrated raspberry solution (T_g'), the freezing curve *AM* was extended to the glass line and designated the intersection point *G* by retaining the equivalent curvature described by Chen model (Kasapis et al., 2000; Rahman et al., 2005). The T_g' was estimated as -47°C and X_s'' was 0.82 kg solids/kg raspberry. Food constituents are in mechanical solid state at the condition of the maximum-freeze-concentration state (Lim & Reid, 1991; Slade & Levine, 1995). The rates of diffusion controlled reactions in frozen foods may decrease considerably at temperatures less than T_g'. At temperatures greater than T_g', the unfrozen matrix becomes less viscous, promoting increased rates of diffusion (Slade & Levine, 1995). The T_g' of raspberries is similar to the T_g' of other fruits (Table 5). The solids in raspberries consist primarily of fructose and glucose and exhibit low T_g'. Ablett et al. (1993) examined the glass transition temperature occurring in fructose solutions and estimated *T_g'* as -48°C. Van den Dries et al. (2000) also studied the relationship between transition in molecular mobility and collapse phenomena in glucose-water systems and reported the *T_g'* of glucose as -53°C.

Evaluating water activity and glass transition concepts for stability

The concept of water activity is an important tool in predicting microbial growth, enzymatic, non-enzymatic activities, and other deteriorative reactions in foods (Rahman and Labuza, 1999). In recent years, the glass transition concept was evaluated to explain selected reaction kinetics in food materials during production and storage (Rahman, 2006). According to the water activity concept, foods are most stable at their monolayer moisture content (Rockland and Nishi, 1980). The glass transition concept suggests that formulations are stable at or below the corresponding glass transition temperature. Water activity relates to the equilibrium condition that establishes a thermodynamic limit to a mechanism, whereas glass formation is a kinetic equilibrium process at temperature below T_g. Scientists related the water activity and glass transition concepts to establish unified stability criteria for foods (Roos, 1993, Bell and Hageman, 1994; Schaller-Povolny et al., 2000; Sablani et al., 2004; 2007b, 2007c). The glass transition temperatures and the water adsorption isotherm were combined to evaluate conditions of storage stability of freeze-dried raspberries (Figure 7 and Table 6). Based on the sorption isotherms, the predictions of the glass transition model underestimate the stable temperature range. For instance, the sorption isotherm at 23°C predicts that freeze-dried raspberries are stable at a BET monolayer water content of 0.056 kg H_2O/kg raspberry or 0.944 kg solids/kg raspberry (Point B in Figure 7). However, at an equivalent solids concentration, the T_g value from the glass line was 3.73°C (point C in Figure 7). Interpretation of the glass transition and water activity data in Table 6 concludes raspberries of 0.973 kg solids/kg raspberry are stable at 23°C or below. Sorption isotherms predicts a water activity of 0.09 at 23°C which, according to this criterion, is lower than monolayer water activity (<0.23) for stable condition of a freeze-dried raspberries. Similar observations were reported by Roos (1993) and Sablani et al., (2007b). The glass transition concept often underestimates the stability temperature for dried fruits containing large concentration of sugars and it overestimates stability temperatures for high molecular weight materials (Sablani et al., 2007b). Detailed studies on physicochemical changes such as anthocyanins, total phenolics concentrations, and antioxidant activities in frozen and dried raspberries stored at a range of water contents/activities may unveil the suitability of water activity or glass transition temperature concepts to predict stability and this research is progressing.

Frozen storage is a common long term storage method adopted for many fresh fruits and vegetables containing freezable water. Storage stability of frozen and dried raspberries may be analyzed by using state diagram, T_g' and T_m' estimated as -47 and -38°C, respectively. The deterioration kinetics of anthocyanins, polyphenolics, vitamins, and other bioactive compounds in raspberries during long term frozen storage can be obtained by storing them at selected temperatures close to their T_g' and T_m'. Fresh raspberries stored below T_g' (< -47°C) may be suitable for long term storage due to minimal chemical and biochemical changes.

Stability of anthocyanin, phenolics and antioxidants in dried raspberries can be determined below, around and above its glass transition temperatures to predict its shelf life. The degradation kinetics of bioactive compounds in raspberries can be related to their corresponding water activities and glass transition temperatures. A relationship between the glass transition temperature and chemical degradation reactions occurring in dried raspberry powder during storage can be established, and may be important in the selection of the suitable storage conditions of dried raspberries.

CONCLUSIONS

The state diagram of raspberries was developed by determining glass line, freezing curve and the conditions of maximally-freeze-concentration. The initial glass transition temperatures of freeze-dried raspberries decreased linearly from 17.5°C to -65.5°C as the total solids content decreased from 0.966 to 0.758 kg dry solids/kg sample. The initial freezing point of freeze-dried raspberries decreased from -2.45°C to -17.4°C as total solids content increased from 0.30 to 0.70 kg dry solids/kg sample. The magnitude of freezing point temperature depression due to increasing total solids was largely due to the presence of glucose and fructose. The state diagram provides an estimate of the characteristics glass transition T_g' and corresponding total solids content X_s'' of -47°C and 0.82 kg solids/kg raspberry, respectively. The quantity of unfreezable water obtained from enthalpy of ice melting and state diagram was comparable. The water adsorption isotherm of freeze-dried raspberries was also constructed. The water sorption data provided the monolayer moisture content values of 0.059 and 0.074 kg H_2O/kg dry raspberry solids in the BET and GAB models, respectively. The state diagram and water adsorption data may be used to predict the stability of anthocyanins, phenolics concentrations and antioxidant activities in low moisture (i.e. dried or with unfreezable water) and high moisture (i.e. containing freezable water) raspberries.

Acknowledgements

This activity was funded, in part, with an Emerging Research Issues Internal Competitive Grant from the Washington State University, College of Agricultural, Human, and Natural Resource Sciences and Agricultural Research Center.

REFERENCES

Ablett, S., Izzard, M. J., Lillford, P. J., Arvannitoyannis, I., and Blanshard, J. M. V. (1993). Calorimetric study of the glass transition occurring in fructose solutions. *Carbohydrate Research,* 242, 13–22.

Bai, Y., Rahman, M. S., and Perera, C. O., Smith, B., and Melton, L. D. (2001). State diagram of apple slices: glass transition and freezing curves. *Food Research International,* 34*,* 89–95.

Bell, L. N. and Hageman, M. J. (1994). Differentiating between the effects of water activity and glass transition dependent mobility on a solid state reaction: aspartame degradation. *Journal of Agricultural and Food Chemistry* 42, 2398-2401

Champion, D., Le Meste, M., and Simatos, D. (2000). Towards an improved understanding of glass transition and relaxations in foods: molecular mobility in the glass transition range. *Trends in Food Science & Technology,* 11*,* 41–55.

Chen, C. S. (1986). Effective molecular weight of aqueous solutions and liquid foods calculated from the freezing point depression. *Journal of Food Science,* 51, 1537–53.

Franks, F. (1986). Metastable water at subzero temperatures. *Journal of Microscopy,* 141(3), 243-249.

Goff, H. D., and Sahagian, M. E. (1996). Glass transitions in aqueous carbohydrate solutions and their relevance to frozen food stability. *Thermochimica Acta*, 280/281, 449–464.

Gordon M., and Taylor J. S. (1952). Ideal copolymers and the second order transitions of synthetic rubbers. I. Non-crystalline copolymers. *Journal of Applied Chemistry,* 2, 493–500.

Greenspan, L. (1977). Humidity fixed points of binary saturated aqueous solutions. *Journal of Research and National Bureau of Standards [A]: Physics and Chemistry,* 81A (1), 89–96.

Janna, O. A., Khairul, A. K., and Maziah, M. (2007). Anthocyanin stability studies in *Tibouchina semidecandra L. Food Chemistry,* 101*,* 1640–1646.

Karel, M., Anglea, S., Buera, P., Karmas, R., Levi, G., and Roos, Y. H. (1994). Stability related transitions in amorphous foods. *Thermochimica Acta,* 246*,* 249–269.

Kasapis, S., Sablani, S. S., Rahman, M. S., Al-Marhubi I. M. and Al-Amri, I. S. (2007). Porosity and the effect of structural changes on the network glass transition temperature. *Journal of Agricultural and Food Chemistry*, 55, 2459–2466.

Kasapis, S., Rahman, M.S., Guizani, N., and Al-Aamri, M. (2000). State diagrams of temperature vs. date solids obtained from the mature fruit. *Journal of Agricultural and Food Chemistry,* 48*,* 3779–3784.

Kerr, W. L., Lim, M. H., Reid, D. S., and Chen, H. (1993). Chemical reaction kinetics in relation to glass transition temperatures in frozen food polymer solutions. *Journal of the Science of Food and Agriculture,* 61, 51–56.

Khalloufi, S., El-Maslouhi, Y., and Ratti, C. (2000). Mathematical model for prediction of glass transition temperature of fruit powders. *Journal of Food Science,* 65(5), 842–848.

Khalloufi, S., and Ratti, C. (2003). Quality deterioration of freeze-dried foods as explained by their glass transition temperature and internal structure. *Journal of Food Science,* 68(3), 892–903.

Labuza, T. P., and Altunakar, B. (2007). Water activity prediction and moisture sorption isotherms. In G. V. Barbosa-Cànovas, A. Fontana, S. Schmidt, T. Labuza (Eds.), *Water activity in Foods: Fundamentals and Applications* (pp 109–154). Blackwell Publishing Ltd.

Lim, L. T., Tang, J. M., and He, J. S. (1995). Moisture sorption characteristics of freeze dried blueberries. *Journal of Food Science,* 60, 810–814

Lim, M. H., and Reid, D.S. (1991). Studies of reaction kinetics in relation to the T_g' of polymers in frozen model systems. In H. Levine, L. Slade (Eds.), *Water Relationship in Foods* (pp. 103–122). Plenum Press, New York.

Rahman M. S. (1995). *Food properties handbook*. Boca Raton, CRC Press FL.

Rahman, M. S. (2006). State diagram of foods: Its potential use in food processing and product stability. *Trends in Food Science and Technology,* 17*,* 129–141.

Rahman, M. S. (2004). State diagram of date flesh using differential scanning calorimetry (DSC). *International Journal of Food Properties,* 7(3), 407–428.

Rahman, M. S., Sablani, S. S., Al-Habsi, N., Al-Maskri, S., and Al-Belushi, R. (2005). State diagram of freeze-dried garlic powder by differential scanning calorimetry and cooling curve methods. *Journal of Food Science,* 70(2)*,* 135–141.

Rahman, M. S., and Labuza, T. P. (1999). Water activity and food preservation. In M. S. Rahman (Ed.), *Handbook of Food Preservation* (pp. 339–382), Marcel Dekker, New York.

Rockland, L. B., and Nishi, S. K. (1980). Influence of water activity on food product quality and stability. *Food Technology,* 34*,* 42–51.

Roos, Y. H. (1997). Frozen state transitions in relation to freeze drying. *Journal of Thermal Analysis,* 48, 535–544

Roos, Y. H. (1992). Phase transitions and transformations in food systems. In R. Heldman, D. B. Lund (Eds.), *Handbook of Food Engineering* (pp. 287–353), Mercel Dekker Inc, New York.

Roos, Y. H., and Karel, M. (1991). Applying state diagrams to food processing and development, *Food Technology,* 45(12), 66–71 and107.

Roos, Y. H. (1993). Water activity and physical state effects on amorphous food stability. *Journal of Food Processing and Preservation,* 16, 433–447.

Roos, Y. H. (1987). Effect of moisture on the thermal behavior of strawberries studied using differential scanning calorimetry. *Journal of Food Science*, 52(1), 146–149.

Sa, M. M., Figueirdo, A. M., and Sereno A. M. (1999). Glass transitions and state diagrams for fresh and processed apple. *Thermochimica Acta,* 329*,* 31–38.

Sa, M. M., and Sereno, A. M. (1994). Glass transitions and state diagrams for typical natural fruits and vegetables. *Thermochimica Acta,* 246*,* 285–297.

Sablani, S. S., Rahman, M.S., Al-Busaidi, S., Guizani, N., Al-Habsi, N., Al-Belushi, R., and Soussi, B. (2007a). Thermal transitions of king fish whole muscle, fat and fat-free muscle by differential scanning calorimetry. *Thermochimica Acta,* 462, 56–63.

Sablani, S. S., Kasapis, S., and Rahman, M. S. (2007b). Evaluating water activity and glass transition concepts for food stability. *Journal of Food Engineering,* 78(1)*,* 266–271.

Sablani, S. S., Al-Belushi, K., Al-Marhubi, I. and Al-Belushi, R. 2007c. Evaluating stability of vitamin C in fortified formula powder using water activity and glass transition *International Journal of Food Properties*, 10: 61-71

Sablani, S. S., Kasapis, S., Rahman, M. S., Al-Jabri, A., and Al-Habsi, N. (2004). Sorption isotherms and state diagram for evaluating stability criteria of abalone. *Food Research International,* 37*,* 915–924.

Sadilova, E., Stintzing, F.C., and Carle, R. (2006). Thermal degradation of acylated and nonacylated anthocyanins. *Journal of Food Science,* 71(8), 504–512.

Schaller-Povolny, L. A., Smith, D. E., and Labuza, T. P. (2000). Effect of water content and molecular weight on the moisture isotherms and glass transition properties of inulin. *International Journal of Food Properties,* 3(2)*,* 173–192.

Silva, M. A., Sobral, P. J. A., and Kieckbusch, T. G. (2006). State diagrams of freeze-dried camu-camu (*Myrciaria dubia* (HBK) Mc Vaugh) pulp with and without maltodextrin addition. *Journal of Food Engineering,* 77*,* 426–432.

Simperler, A., Kornherr, A., Chopra, R., Bonnet, P.A., Williams, J., Motherwell, W. D. S., and Zifferer, G. (2006). Glass transition temperature of glucose, sucrose and trehalose: an experimental and in silico study. *Journal of Physical Chemistry,* 110*,* 19678–19684.

Slade, L., and Levine, H. (1991). Beyond water activity - Recent advances based on an alternative approach to the assessment of food quality and safety. *Critical Reviews in Food Science and Nutrition,* 30 (2–3)*,* 115–360

Sopade, P. A., Bhandari, B. R., D'Arcy, B., Halley, P., and Caffin, N. (2002). A study of vitrification of Australian honeys at different moisture contents. In H. Levine (Ed.), *Amorphous Food and Pharmaceutical Systems* (pp. 169–183), The Royal Society of Chemistry, Cambridge.

Tang, J., Sokhansanj, S., Yannacopoulos, S., and Kasap, S. O. (1991). Specific heat capacity of lentil seeds by differential scanning calorimetry. *Transaction of the American Society of Agricultural Engineering,* 34, 517–522

Telis, V. R. N., and Sobral, P. J. A. (2001). Glass transitions and state diagram for freeze dried pineapple. *Lebensmittel-Wissenschaft und-Technologie-Food Science and Technology,* 34*,* 199–205.

Telis, V. R. N., and Sobral, P. J. A. (2002). Glass transitions for freeze dried and air dried tomato. *Food Research International,* 35, 435–443.

Telis, V. R. N., Sobral, P. J. D., and Telis-Romero, J. (2006). Sorption isotherm, glass transitions and state diagram for freeze-dried plum skin and pulp. *Food Science and Technology International,* 12(3)*,* 181–187.

Van den Dries, I. J., Besseling, N. A. M., Dusschoten, D. V., Hemminga, M. A., and Linden, E. V. D. (2000). Relation between a transition in molecular mobility and collapse phenomena in glucose-water systems. *Journal of Physical Chemistry,* 104*,* 9260-9266

Wang, S. Y., and Lin, H. S. (2000). Antioxidant activity in fruits and leaves of blackberry, raspberry, and strawberry varies with cultivar and developmental stage. *Journal of Agricultural and Food Chemistry,* 48*,* 140–146.

Wang, H., Zhang, S., and Cuen, G. (2008). Glass transition and state diagram for fresh and freeze-dried Chinese gooseberry. *Journal of Food Engineering,* 84, 307–312.

Zhang, Y., Vareed, S. K., and Nair, M. G. (2005). Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. *Life Sciences*, 76, 1465–1472.

LEGENDS TO FIGURES

Figure 1 Water adsorption isotherm of freeze dried raspberry powders at 23^{o}C.

Figure 2 Glass transition temperatures of freeze-dried raspberry powders equilibrated over selected water activities (scan rate 5^{o}C/min)

Figure 3 Effect of DSC scanning rate on glass transition temperatures (water content of rapsberry = 0.034 kg water/kg raspberry).

Figure 4 Typical thermogram of raspberry powder containing freezable water (0.30 kg water/kg raspberry) (scan rate 5^{o}C/min)

Figure 5 Change in enthalpy of ice melting as a function of water content in raspberries

Figure 6 State diagram of raspberries (*AM*: freezing point curve modeled using Chen equation; *DE* glass line modeled using Gordon-Taylor equation; *M*': end point of freezing; *G*: glass transition of maximal-freeze-concentration).

Figure 7 Variation of glass transition temperature, water activity with water content of freeze-dried raspberries.

Table 1. Model parameters of water adsorption isotherms of freeze-dried raspberries, strawberries, blueberries, glucose and fructose

Models Constants		Raspberry[a] (23°C)	Raspberry[b] (25°C)	Strawberry[b] (30°C)	Blueberry[b] (25°C)	Blueberry[c] (4 to 45°C)	Glucose[d]	Fructose[d]
GAB	M_g	0.074	0.109	0.107	0.113	0.174	NA	NA
	C	5.53	7.57	1.95	1.76	0.005	NA	NA
	K	0.904	1.02	0.98	0.96	1.12	NA	NA
	R^2	0.996	NA	0.858	NA	NA	NA	NA
GT	k	4.73	2.85	4.29	4.02	NA	4.52	3.76
		42.62	47.8	34.2	22	NA	36	10
T_{gs}		0.930	NA	NA	NA	NA	NA	NA
	R^2							

[a]Current Study; [b]Khalloufi et.al (2000); [c]Lim et al. (1995); [d]Roos (1993); NA = Not available

Table 2. Glass transition temperatures (initial, T_{gi}, mid, T_{gm} and end-points, T_{ge}) of raspberries influenced by water content (above unfrozen water content i.e. no ice formation, scan rate 5°C/min).

X_s (kg solids/kg raspberry)	X_w (kg water/kg raspberry)	T_{gi} (°C)	T_{gm} (°C)	T_{ge} (°C)
0.966	0.034	17.5 (0.9)[a]	19.2 (1.0)	22.4 (0.5)
0.954	0.046	7.31 (0.8)	9.54 (0.7)	12.7 (0.1)
0.931	0.069	-5.03 (1.8)	-4.2 (2.2)	1.12 (4.8)
0.914	0.086	-12.0 (4.6)	-11.2 (5.5)	-4.23 (6.0)
0.887	0.112	-19.4 (6.3)	-16.3 (6.1)	-9.85 (5.8)
0.866	0.134	-29.7 (6.4)	-28.7 (6.4)	-24.4 (10.0)
0.825	0.175	-57.0 (0.5)	-53.9 (1.6)	-51.8 (1.6)
0.758	0.242	-65.5 (4.5)	-62.1 (4.4)	-59.4 (4.3)

[a] Standard deviation of 3 replicates.

Table 3. Influence of annealing time on the glass transition temperature (Raspberries with water content of 0.4 kg H_2O/kg raspberry).

Annealing time (^oC/min)	T_{gi} (^oC)	T_{gm} (^oC)	T_{ge} (^oC)
0	-61.2	-56.0	-55.4
30	-62.3	-59.4	-55.9
60	-62.8	-58.2	-55.6

Table 4. Solid contents (X_s), initial freezing point (T_F) and enthalpy (ΔH_m) of ice melting determined with DSC

X_s (kg solids/kg raspberry)	T_F (°C)	T_g' (°C)	T_m' (°C)	ΔH_m (kJ/kg)
0.3	-2.45 (0.2)[a]	-57.4 (2.6)	-19.4 (1.0)	94.1 (7.7)
0.4	-7.62 (1.6)	-55.8 (1.0)	-25.2 (3.7)	79.7 (21.5)
0.5	-8.02 (0.9)	n.d.	-36.5 (5.6)	70.8 (6.1)
0.6	-12.5 (1.9)	n.d.	-40.7 (5.2)	32.4 (19.5)
0.7	-17.4 (4.9)	n.d.	-36.5 (3.1)	19.6 (5.3)

[a] Standard deviation of 3 replicates, n.d. = not detectable

Table 5. Comparison of T_g' value of raspberries with T_g' of selected fruits

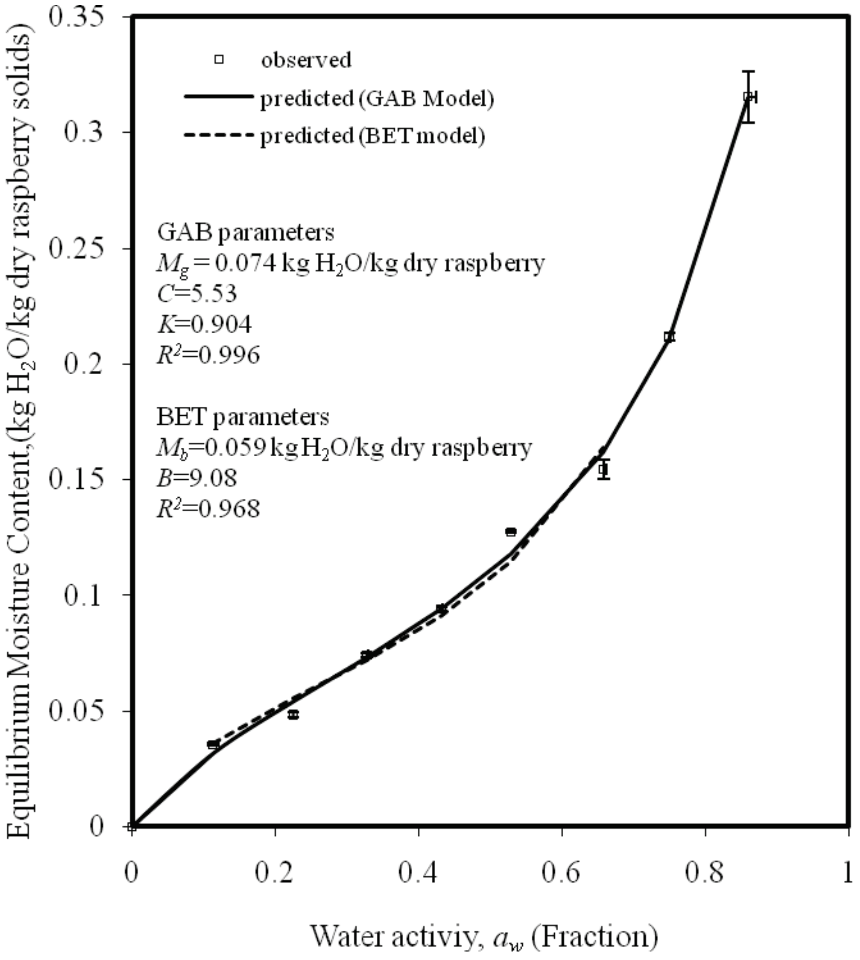
Product	T_g' (°C)
Freeze dried Raspberries[a]	-48
Freeze-dried pineapple[b]	-51.6
Freeze dried and osmoticaly dehydrated apples[c]	-71.1 (For freeze dried sample) -61.5 (For osmoticaly dried sample).
Dried apple slices[d]	-55.1
Freeze dried plums[e]	-57.5
Fresh and freeze dried Chinese gooseberries[f]	-57.2

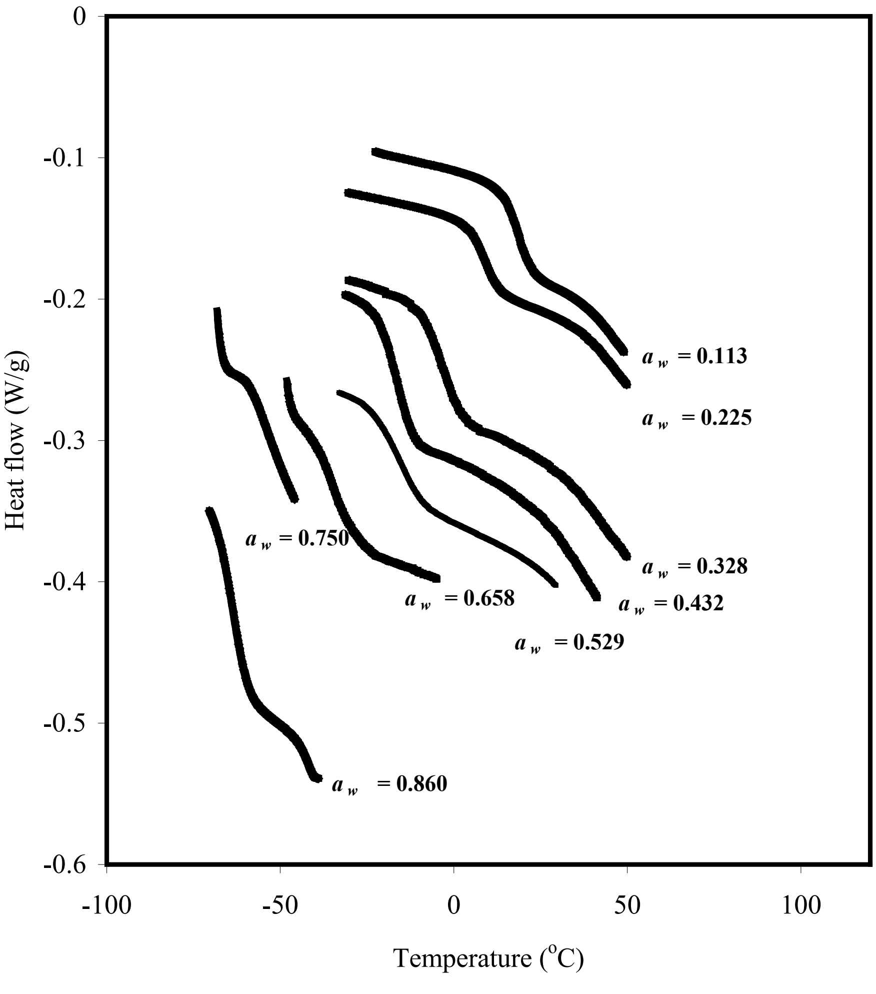
Date flesh[g]	-46.4
Freeze dried Camu Camu[h]	-58.8 for natural pulp and -40.1 for camu camu with 30% maltodextrin DE 20 addition.
Onions, Grapes and Strawberries[i]	For Onion, -58.3 For grape, -50.3 For strawberry, -50.1

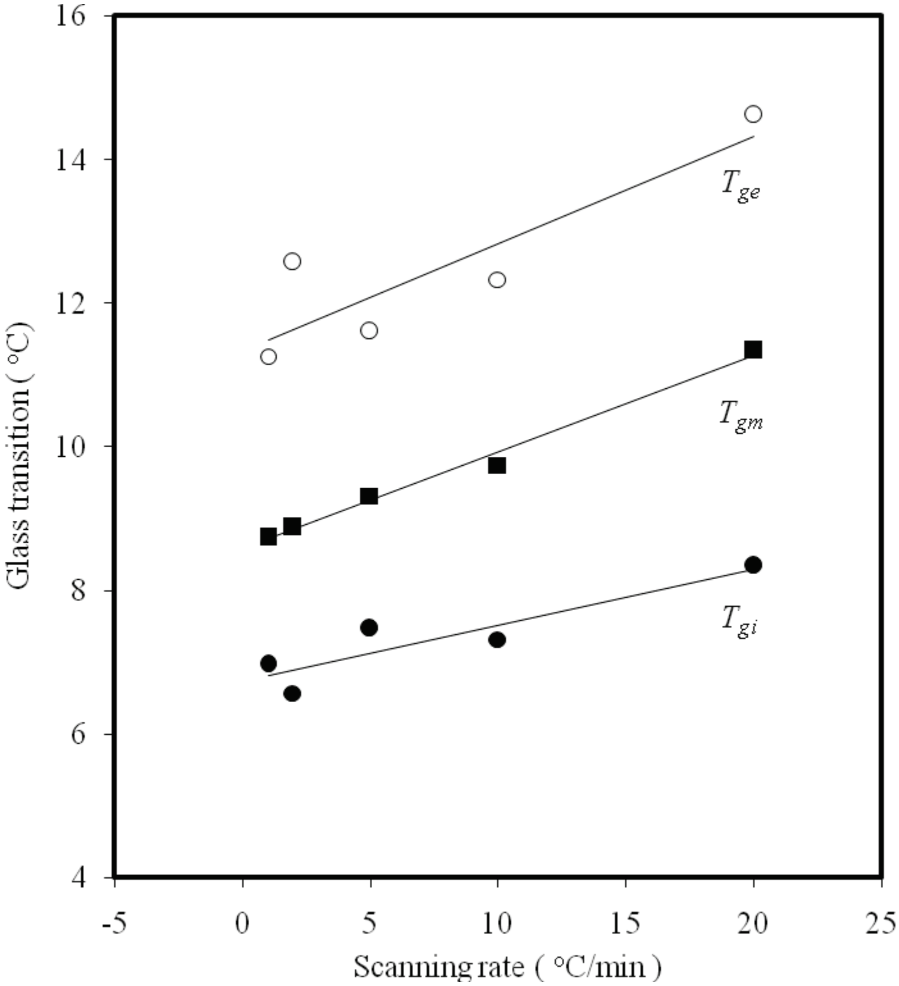
[a]Current study; [b]Telis and Sobral, (2001); [c] Sa et.al, (1999); [d]Bai et.al, (2001); [e]Telis et.al, (2006); [f]Wang et.al, (2008); [g]Rahman, (2004); [h]Silva et.al, (2006); [i]Sa and Sereno, (1994).

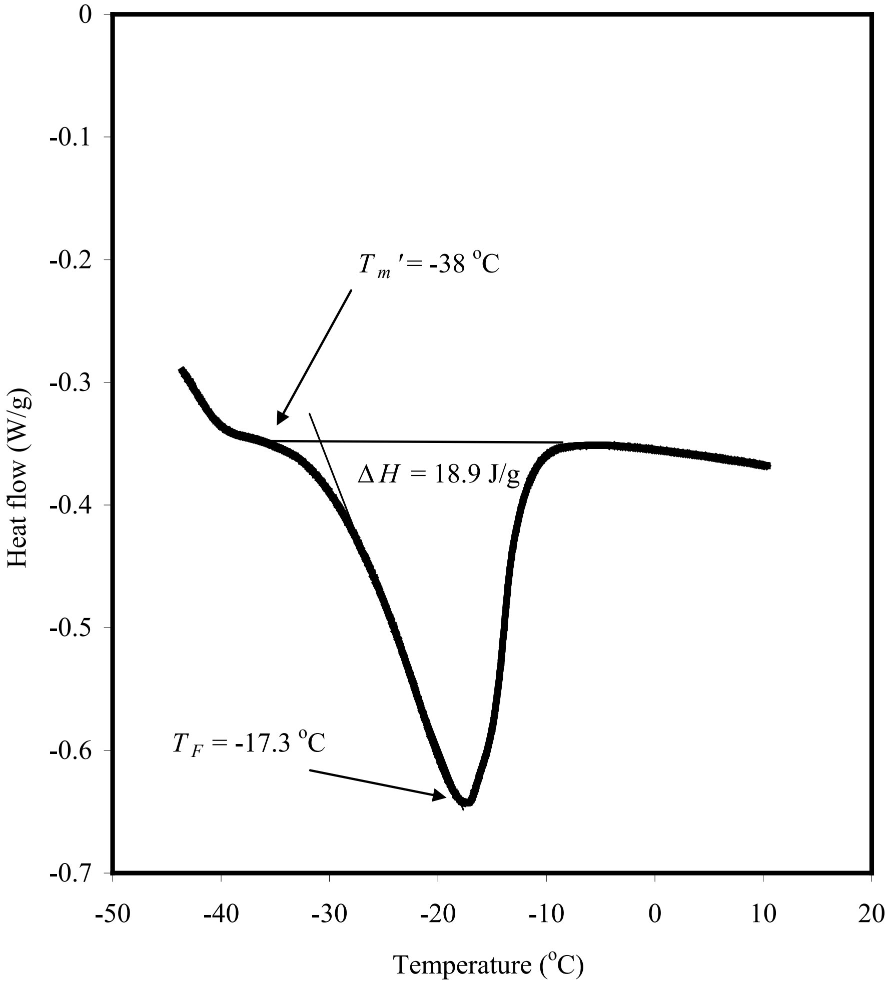
Table 6. Evaluating water sorption isotherm and glass transition models of freeze-dried raspberries

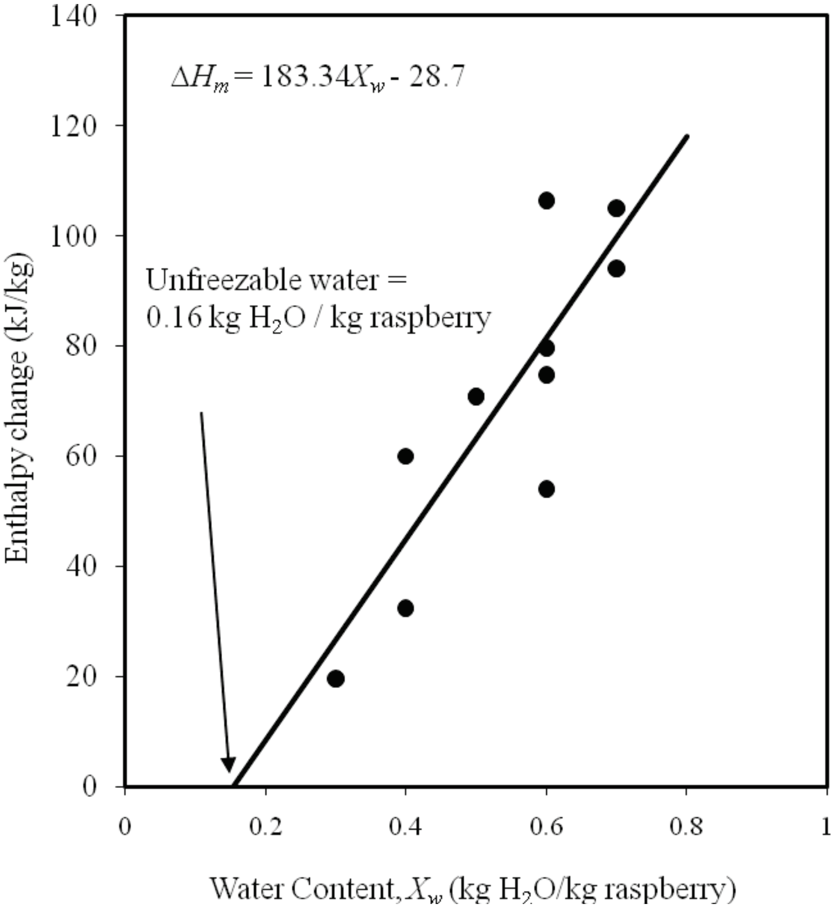
Sorption isotherm model				Glass transition model		
Temperature (°C)	Monolayer water content (from BET model) (kg water/kg rapsberry)	a_w corresponding to the monolayer water content (fraction)	T_g from the glass transition model (°C)	T_g (°C)	Water content (kg water/kg rapsberry)	a_w at corresponding water content (fraction)
23	0.056	0.23	3.73	23	0.027	0.09

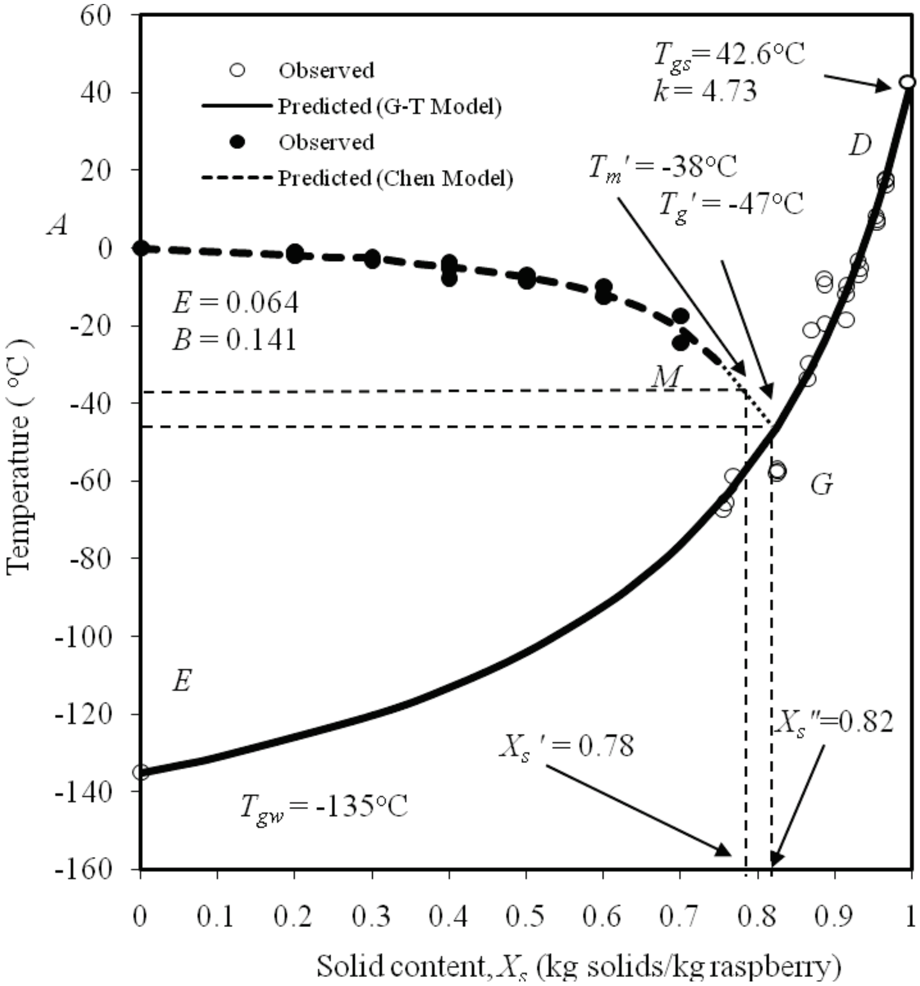

observed
predicted (GAB Model)
predicted (BET model)
GAB parameters
M_g = 0.074 kg H_2O/kg dry raspberry
C=5.53
K=0.904
R^2=0.996
BET parameters
M_b=0.059 kg H_2O/kg dry raspberry
B=9.08
R^2=0.968
Equilibrium Moisture Content,(kg H_2O/kg dry raspberry solids)
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0
0.2
0.4
0.6
0.8
1
Water activiy, a_w (Fraction)

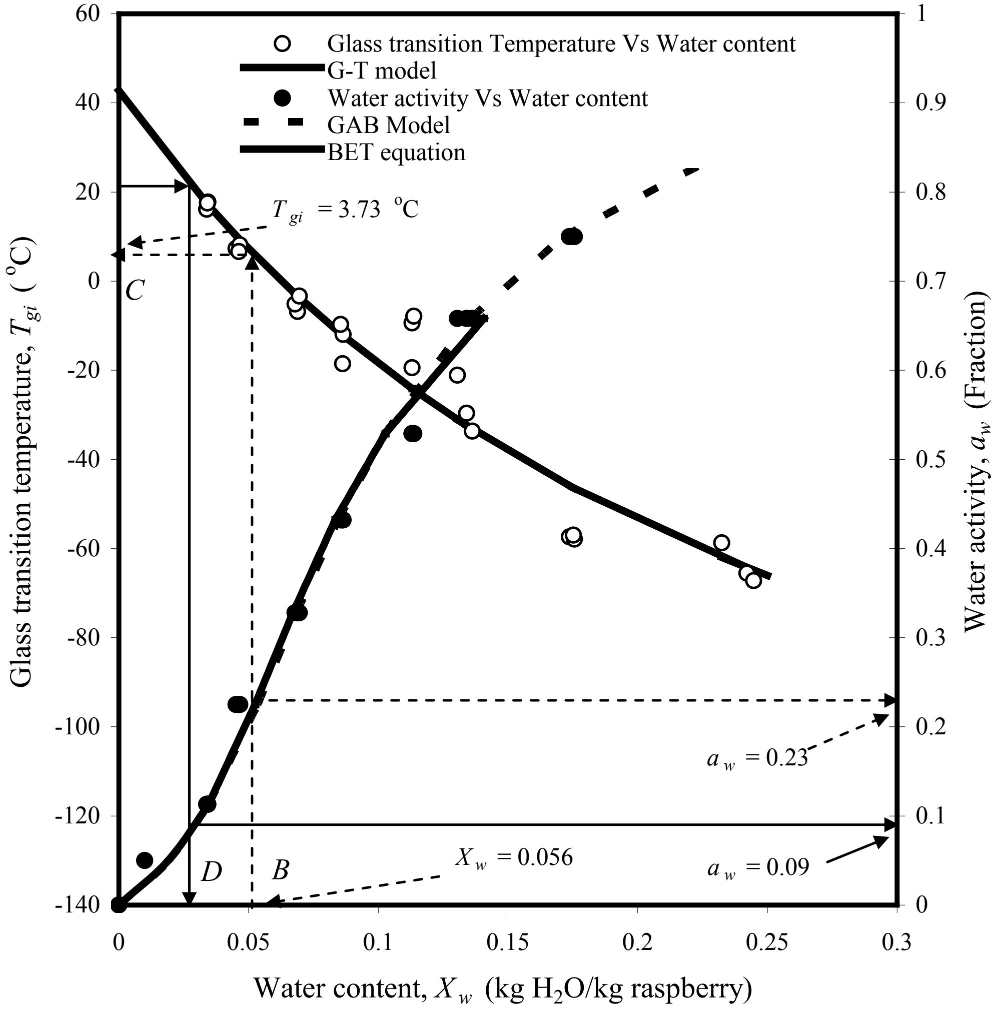

0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
Heat flow (W/g)
-100
-50
0
50
100
Temperature (°C)
a w = 0.113
a w = 0.225
a w = 0.328
a w = 0.432
a w = 0.529
a w = 0.658
a w = 0.750
a w = 0.860


Glass transition (°C)
Scanning rate (°C/min)
16
14
12
10
8
6
4
-5
0
5
10
15
20
25
T_{ge}
T_{gm}
T_{gi}


0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.6
-0.7
Heat flow (W/g)
-50
-40
-30
-20
-10
0
10
20
Temperature (°C)
T_m' = -38 °C
ΔH = 18.9 J/g
T_F = -17.3 °C


$\Delta H_m = 183.34X_w - 28.7$
Unfreezable water =
0.16 kg H_2O / kg raspberry
Enthalpy change (kJ/kg)
0
20
40
60
80
100
120
140
0
0.2
0.4
0.6
0.8
1
Water Content, X_w (kg H_2O/kg raspberry)


60
40
20
0
-20
-40
-60
-80
-100
-120
-140
-160
Temperature (°C)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
Solid content, X_s (kg solids/kg raspberry)
○ Observed
Predicted (G-T Model)
● Observed
Predicted (Chen Model)
T_{gs}= 42.6°C
k= 4.73
D
T_m' = -38°C
T_g' = -47°C
A
E = 0.064
B = 0.141
M
G
E
X_s' = 0.78
X_s''=0.82
T_{gw} = -135°C


Glass transition Temperature Vs Water content
G-T model
Water activity Vs Water content
GAB Model
BET equation
T_{gi} = 3.73 °C
C
D
B
X_w = 0.056
a_w = 0.23
a_w = 0.09
Glass transition temperature, T_{gi} (°C)
Water activity, a_w (Fraction)
Water content, X_w (kg H_2O/kg raspberry)
60
40
20
0
-20
-40
-60
-80
-100
-120
-140
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
0.05
0.1
0.15
0.2
0.25
0.3