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Thermal characteristics of *Agaricus bisporus* mushroom: freezing point, glass transition, and maximal-freeze-concentration condition

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Abstract

The stability of foods strongly depends on the state of water (i.e. water activity) and temperature. *Agaricus bisporus* mushroom plays an important role as a nutritional and functional food; however little information is available on the effect of processing on its stability. This study measures the thermal characteristics and sorption isotherm of Agaricus bisporus by differential scanning calorimetry (DSC) and isopiestic method, respectively. Thermograms of samples containing un-freezable water (below moisture content 0. 11 g/g sample, i.e. wet basis) showed no glass transition which is indicative of the complexity of mushroom texture. Samples containing freezable water above 0.17 g/g sample exhibited glass transition. The BET monolayer value was 0.061 g/g dry-solids (i.e. dry basis). Actual maximal-freeze-concentration conditions was found as X_s' (characteristic solids content) = 0.782 g/g sample, and T_m' (characteristic end point of freezing) = -30°C. The glass transition data and isotherm of *A. bisporus* containing un-freezable water could be used to determine stability region of dried mushroom during its storage, whereas T_m' to determine the stability for the frozen storage. In addition it could be used in designing drying and freezing processes, respectively.

Introduction

Health consciousness is one of the dominant drivers of consumer markets in the 21st century. Functional food products are a new category of foods that are marketed for health benefits or functionality. A food can be regarded as functional if it has been scientifically proven to have special health benefits in addition to providing basic nutrients and nutritional benefits (Guizani and Sablani, 2007). Current research is now directed towards finding naturally occurring antioxidants particularly of plant origin (Emynur Shafekh *et al*., 2012). Mushrooms are recognized as a nutritious food with preferred flavor and texture as well as an important source of biologically active compounds with medicinal value. They are an important source of nutrients and of a variety of secondary metabolites, including various phenolic compounds, known for their excellent antioxidant activities (Mau *et al*., 2002). Various antioxidant compounds are used widely in different food products that help in providing protection to oxidative damages by free-radical molecules (Kumari *et al*., 2011). In recent years, the consumption of mushrooms has risen greatly due to continuous developments in cultivation, harvest, postharvest processing and storage treatments. *Agaricus bisporus* (*A. bisporus*) is one of the most commonly consumed mushrooms in the world including Oman. *A. bisporus* is an excellent source of several essential amino acids, vitamins (B2, niacin and folate) and minerals (potassium, phosphorus, zinc and copper) (Manzi *et al*., 2001). However, the commercial value of *A. bisporus* can be decreased or fully lost within few days if it is stored at ambient temperature.

Along with its consumption as fresh, *A. bisporus* can be preserved in the dried, frozen and freeze dried forms. Dehydration is one of the most important preservation methods employed in storage of mushroom and dehydrated mushrooms have been used as ingredients in food preparation to give flavor and mouth-feel and as functional foods. The major objective in drying is the reduction of moisture content to a certain level food depending on the type of food, which allows safe storage and preservation (Seiiedlou *et al.*, 2010). However drying methods play an important role in production of the dried vegetables and the bioactive compounds and their antioxidant capacity might be lost during drying process (Hung and Duy, 2012). The bioactive compounds of mushroom and their antioxidant capacity might as well be lost during the preservation

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process and the storage period. The biochemical and microbial stabilities of foods strongly depends on the state of water related to the water activity. Moisture sorption isotherms represent the equilibrium relationship between water activity and moisture content of foods at constant pressure and temperature. A sound knowledge of the relationship between moisture content and equilibrium relative humidity is essential in the formulation of foods and in their storage stability (Tsami *et al*., 1999).

Thermal analysis determines different phases and states of foods as a function of water content and temperature (Rahman, 2004; 2006). The state diagram, a plot of thermal characteristic temperatures as a function of solids content, is used to clearly visualize food's characteristics (Rahman, 2009). It has been reported in the literature that food can be considered very stable at its glassy state. Glass transitions of pure components are more commonly reported in the literature, than the real foods, which are more complex multi-components mixtures. Molecular mobility of glassy materials is significantly reduced below its glass transition. This in turn delays various deteriorative changes such as texture loss, enzymatic spoilage and flavor loss in foods during storage (Mitchell, 1998). Studies by Rahman *et al.* (2005) and Sablani *et al.* (2007) indicated that the concept of glass transition should be added along with the existing concept of water activity, to get a better understanding about the factors governing the stability of foods. The glass transition temperature values of dates (Kasapis *et al.*, 2000; Rahman, 2004; Guizani *et al.*, 2010), tuna (Rahman *et al.*, 2003), king fish muscle (Sablani *et al*., 2007), strawberry (Roos, 1987), garlic (Rahman *et al*., 2005) and grapefruit (Fabra *et al*., 2009) were presented in the literature. However, the characteristic maximal-freeze-concentration conditions (T_m' and T_g'") of foods are relatively scarce in the literature (Haiying *et al*., 2007).

Although research was focused on the therapeutic effects and antioxidant properties of mushrooms, little information is available about their structural properties in relation to water activity and thermal characteristics. The overall objective of this study was to measure the glass transition, freezing curve (freezing point versus solids content), and maximal-freeze-concentration conditions (T_g'", T_m' and X_s') and other related characteristics of *A. bisporus*, a widely cultivated edible mushroom. In addition moisture sorption isotherm was measured. These data could be used in determining the storage stability of dried and frozen mushroom.

Material and Methods

Samples preparation

Samples of one kilogram *A. bisporous* mushroom were collected from Gulf Mushroom Products (Barka, Oman) and transported to the laboratory on the same day of harvest. Mushroom samples were frozen at -40°C for 12 hr and freeze dried in freeze dryer for 3 days at 20°C (i.e. sublimation temperature) with a pressure of 200 Pa. The condenser temperature for air was at -60°C. The dried samples were crushed to a fine powder using mechanical grinder and stored at -40°C until used for chemical and thermal analyses.

Chemical composition

Samples were analyzed for chemical composition (moisture, protein, fat, carbohydrates, and ash) using AOAC (1995) procedures. The moisture content was determined by drying 1 g of the samples in a thermostatically controlled oven at 105°C for at least 18 hr. The crude protein content (N×4.38) was estimated by the Macro Kjeldahl method. Fat was determined by extracting with petroleum ether in a Soxhlet apparatus and ash content was determined by incineration of freeze-dried powder at 550°C for 24 hours. Total carbohydrates were calculated by the difference from protein, fat and ash on a dry basis. Total polyphenol were measured by the Folin-Ciocalteu assay of Singleton and Rossi (1965). 10 µl of *A. bisporous* extract was taken in a test tube and 3 ml of distilled water followed by 250 µl Folin-Ciocalteu reagent were added to it and vortexed. After a short incubation of 5 min, 750 µl of sodium carbonate (1.9 M) and 990 µl of water were added to make up the total volume up to 5 ml. The final mixture was incubated for 2 h. The absorbance at 765 nm was measured and compared with gallic acid standards. The concentration of phenolic compounds in mushroom extracts was expressed as gallic acid equivalents (GAE). All the measurements were taken in triplicate and the mean values were calculated.

Flavonoids were determined according to the aluminum chloride colorimetric assay of Kim *et al.* (2003). Distilled water (4 ml) was added to 1 ml of *A. bisporous* extract. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). The mixtures were incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide were added. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink color developed was determined at 510 nm. A

calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CEQ)/100 g sample. All the measurements were taken in triplicate and the mean values were calculated.

Preparation of equilibrated samples

Mushroom samples (approximately 5 g) were placed in glass bottles with opened caps and stored in air-sealed glass jars at 20°C. The jars were maintained at different relative humidity with saturated salt solutions placed in beakers. The salts used were: LiCl, CH_3COOK, $MgCl_2$, K_2CO_3, NaBr, $SrCl_2$, KCl, and K_2SO_4 (BHD, Poole, England). Relative humidity values for these solutions were obtained from the compilation of Spiess and Wolf (1987). The samples were equilibrated until a constant mass was achieved. A 5 ml beaker containing thymol was placed inside the jars at higher water activity to prevent mold growth during storage (Guizani *et al*., 2008). The equilibration took around 6 weeks. The equilibrated samples (water content: 0.046-0.107 g/g sample) in closed caped bottles were stored at -20°C until used for thermal analysis. Samples at high moisture content (water content: 0.300-0.950 g/g sample) were prepared by mixing freeze dried powder with predetermined water. The moisture content of equilibrated samples was measured by oven drying method at 105°C for at least 18 h.

Moisture sorption isotherm

The water sorption isotherm was modeled by widely used BET and GAB models. The BET equation is (Brunauer *et al*., 1938):

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

Where, M_b is the BET monolayer moisture content (g/g dry solids) and B is a constant related to the net heat of sorption, respectively. The BET isotherm holds well between water activities of 0.05 and 0.45, an adequate range for the calculation of parameters M_b and B (Labuza, 1968). Equation 1 can be linearized (y versus x) and plotted as $[a_w/(1-a_w)M_w]$ versus a_w. The values of B and M_b can be calculated from the slope (i.e. $(B-1)/M_bB$) and intercept (i.e. $1/M_bB$) of the best fitted linear line, considering water activity vales only up to 0.45. The BET model parameters were first determined based on the linearized Eq. 1. In addition SAS non-linear regression procedure was also used to determine the parameters (SAS, 2001). Anderson (1946) modified BET equation for multi-layers for strengthening the physical meaning and the fitting ability of the model. The GAB equation is one of the most popular and widely used for foods, and the equation is:

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

Where, M_g is the GAB monolayer moisture content (g/g dry-solids), C is a constant related to the monolayer heat of sorption and K is a factor related to the heat of sorption of the multilayer water, respectively. The model parameters were estimated using SAS non-linear regression (SAS, 2001).

Differential scanning calorimetry (DSC)

The freezing point and glass transition of mushroom at different moisture content were measured by Differential Scanning Calorimetry (DSC Q10, TA Instruments, New Castle, DE, USA). Mechanical refrigerated cooling system was used to cool the sample up to -90°C. The instrument was calibrated for heat flow and temperature using distilled water (m.p. 0◦C; ΔH_m 334 J/g), and indium (m.p. 156.5◦C; ΔH_m 28.5 J/g). Aluminum pan of 30 ml, which could be sealed with lid were used in all experiments with an empty sealed pan as reference. Nitrogen, at a flow rate of 50 ml/min, was used as a carrier gas.

Samples (containing un-freezable water) of 5-10 mg were placed in an aluminum pan and then sealed. The sealed pan with sample was cooled to -90°C at 5°C/min, and equilibrated for 10 min. After equilibration, it was scanned from -90°C to 100°C at a heating rate of 10°C/min. In the case of samples without freezable water, glass transition was determined from a shift in the thermogram line. The glass transition was analyzed for the onset, mid and end of glass transition; and the change in specific heat at the transition. Selected data were replicated 3-4 times.

A different procedure was used for samples containing high water (moisture content: 0.30-0.95 g/g sample) having freezable water. Samples of 5-10 mg of the powder in a sealed aluminum pan were cooled to -90°C at 5°C/min and equilibrated for 10 min. The sample was then scanned from -90°C at 10°C/min to 100°C in order to determine freezing point and apparent maximal-freeze-concentration condition $[(T_m')_a$ and $(T_g''')_a]$. Sample containing 0.400 g moisture/g sample was used to determine annealed maximal-freeze-concentration and ultimate freeze-concentration conditions. In this case, after knowing the apparent $(T_m')_a$ and $(T_g''')_a$, samples (moisture content: 0.400 g/g sample) were scanned similarly with 30 min annealing at $[(T_m')_a$ -1] °C, in order to measure actual $(T_m')_n$ and $(T_g''')_n$. The use of annealing condition allowed to maximize the formation of ice before second heating cycle and to

avoid the appearance of exothermic or endothermic peak before the glass transition (Rahman *et al*., 2010). The initial or equilibrium freezing point was considered as the point of maximum slope of the endothermic peak. For the materials showing wide peak of ice melting on the DSC thermogram, the point of maximum slope corresponds well with the initial freezing point estimated from established cooling curve method (Rahman, 2004). This point was also calibrated with freezing point of distilled water. The latent heat of ice melting (or freezing) was estimated from the area of the ice melting endotherm.

Freezing point prediction model

Freezing point was fitted into Chen (1986) model, which is based on Clausius-Clapeyron equation. The Clausius-Clapeyron equation is limited to the ideal solution (i.e. for a very dilute solution), which can be improved by introducing parameters for non-ideal behavior when fraction of total water is unavailable for the formation of ice. The un-freezable water content B can be defined as the ratio of unfrozen water to the solids. Considering this concept, Chen (1986) extended the Clausius-Clapeyron equation by introducing a parameter B as:

$$\delta = -\frac{\beta}{\lambda_w}\ln\left[\frac{1-X_S^0-BX_S^0}{1-X_S^0-BX_S^0+EX_S^0}\right] \qquad (3)$$

where δ is the freezing point depression (T_w-T_F), T_F is the freezing point of food (°C), Tw is the freezing point of water (°C), β is the molar freezing point constant of water (1860 kg K/kg mol), λ_w is the molecular weight of water, X_s^o is the initial solids mass fraction before freezing (g/g sample), E is the molecular weight ratio of water and solids (λ_w/λ_s) and λ_s is the molecular weight of solutes, respectively. The model parameters E and B were estimated using non-linear regression procedure in SAS (2001).

Results and Discussion

Chemical composition

The average chemical composition of *A. bisporous* mushroom is shown in Table 1. Carbohydrates followed by proteins contribute mostly to the solids of *A. bisporous* with approximately 0.53 and 0.35 g/g sample, respectively. The carbohydrates content of *A. bisporous* is similar to previously reported values for edible mushrooms. The carbohydrate contents of some wild edible mushrooms were reported to be between 0.41 and 0.65 g/g sample (Sanmee *et al*., 2003). Colak *et al.* (2007) found carbohydrate contents of *M. mastoidea*, *L. nuda*, *H. excipuliformis* and *A. rubescens* as 0.558, 0.419, 0.628, 0.297 and 0.653, respectively. Kalmis *et al.* (2011) reported that the carbohydrate content of *T. terreum*, *T. portentosum*, *T. portentosum* and *T. giganteum* were as 0.311, 0.340, 0.523 and 0.701 g/g dry-solids, respectively. For proteins Diez and Alvarez (2001) reported lower values for *T. portentosum* and *T. terreum*, 0.196 and 0.201 g/g sample, respectively. Yildiz *et al.* (2005) reported a higher amount of protein for some *Tricholoma* species: 0.465 g/g sample for *T. terreum*, and 0.505 g/g sample for *T. ustale*. Barros *et al.* (2007) reported that *T. portentosum* contained protein as 0.305 g/g dry-solids.

Table 1. Chemical analysis of freeze dried *A. bisporous*

Composition	Values
Moisture Content (g/g sample)	0.144 ± 0.004
Crude Fat (g/g dry-solids sample)	0.029 ± 0.003
Crude Protein (g/g dry-solids sample)	0.346 ± 0.003
Ash Content (g/g dry-solids sample)	0.095 ± 0.009
Carbohydrate* (g/g dry-solids sample)	0.529 ± 0.005
Total polyphenols (Gallic Acid Equivalents, GAE mg/g dry-solids sample)	19.8 ± 0.4
Flavonoids (Catechin Equivalents mg/g dry-solids sample)	0.76 ± 0.05

*Carbohydrate was estimated from the difference of fat, protein, and ash on dry basis

The phenolics contents of *A. bisporous* were 19.79 mg GAE/g dry-solids sample. These values are higher than those reported by Palcios *et al*. (2011) for eight types of edible mushrooms (*Agaricus bisporus*, *Boletus edulis*, *Calocybe gambosa*, *Cantharellus cibarius*, *Craterellus cornucopioides*, *Hygrophorus marzuolus*, *Lactarius deliciosus* and *Pleurotus ostreatus*), which ranged between 1 and 6 mg GAE/g dry-solids sample. The flavonoids content of A. bisporus were 0.76 mg CEQ/g dry-solids sample, lower than the flavonoid concentrations of eight types of edible mushrooms studied by Palacios *et al.* (2011), which ranged between 0.9 and 3.0 mg CEQ/g dry-solids.

Sorption isotherm

Figure 1, plot of equilibrium moisture content versus water activity, shows the adsorption isotherms of the freeze-dried mushroom. The isotherm data was fitted to BET and GAB models. The BET-monolayer (i.e. M_b) values for freeze-dried mushroom was 0.061 g/100 g dry-solids, and the model parameter relating the binding energy (i.e. *B*) was 19.06. These values were estimated based on the linearized graphical procedure. The non-linear regression approach using SAS, provided the values of M_b and *B* as 0.063 g/g dry-solids and 16.73, respectively ($p < 0.02$). The initial values from the linearized method were used to optimize the non-linear regression. The values for *A. bisporous* mushroom were comparable to those for Shiitake mushroom (Ko *et al*., 1999). GAB monolayer

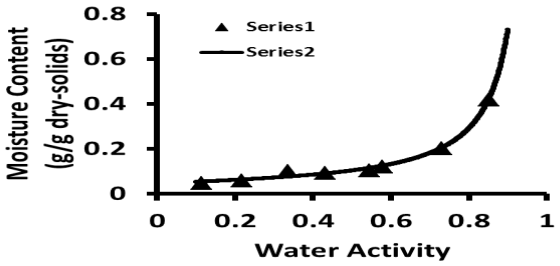

Figure 1. Moisture adsorption isotherm of *A. bisporous* at 20°C (series1: experimental points, series2: GAM model line)

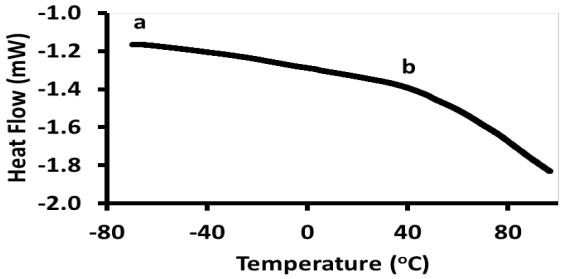

Figure 2. DSC thermogram line of *A. bisporous* equilibrated at relative humidity 11.3% with no freeze-able (X_w: 0.046 g/g sample)

values for Shiitake mushroom were between 0.054 and 0.068 g water/g dry-solids, 0.062 and 0.073 g water/g dry-solids, and 0.065 and 0.073 g water/100 g dry-solids for hot-air dried, vacuum-dried, and freeze-dried Shiitake mushrooms, respectively. The BET-monolayer is an effective method for estimating the amount of water bound to specific polar sites in dried foods or biomaterials. The whole range of isotherms up to a water activity of 0.9 could be predicted using the GAB model. The model parameters Mg, C and K were estimated as 0.050 g/g dry-solids, 108.7 and 1.035 for freeze-dried mushroom powder ($p < 0.001$). The BET monolayer is commonly used to determine the most stable conditions for foods, and GAB monolayer is used to predict the isotherm up to a water activity of 0.9 for use in drying process and packaging design.

Thermal transitions of mushroom containing un-freezable water

Figure 2 shows a typical DSC thermogram line of freeze dried mushroom containing un-freezable water. This indicates no trace of glass transition (i.e. no shift or change in slope). Similar behavior was also observed for the samples containing moisture from 0.046 to 0.107 g/g sample. This limitation of the DSC method was also identified earlier in the case of multi-components complex foods or biomaterials and thoroughly discussed by Rahman and Al-Saidi (2010). The low moisture content samples made more complexity. It is important to mention that for

Table 2. Glass transition of the samples containing un-freezable water

X_w (g/g sample)	T_{gi} (°C)	T_{gp} (°C)	T_{ge} (°C)	ΔC_p (J/kg K)
0.167	-1.5	6.3	20.5	443
0.295	-25.3	-15.7	-5.9	792

X_w :characteristic solid content.
T_{gi}, T_{gp}, T_{ge} : initial, mid and end of glass transition temperature.
ΔC_p: Change in specific heat at the transition (kJ/kg K).

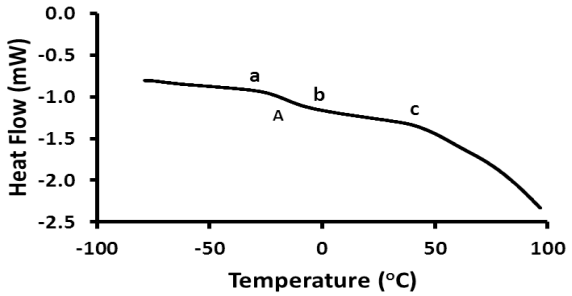

Figure 3. DSC thermogram line of *A. bisporous* equilibrated at relative humidity 85.1% with no freezable water (X_w: 0.295 g/g sample) A: glass transition, a: onset of glass transition, b: end of glass transition, c: onset of melting

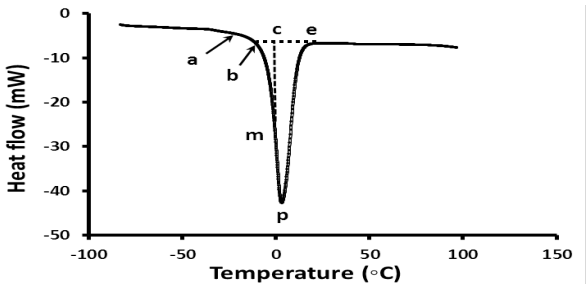

Figure 4. DSC thermogram of *A. bisporous* containing freezable water (X_w: 0.900 g/g sample), a: T_g'''(°C): maximal-freeze concentration condition), b: T_m' (°C): characteristic temperature of end point of freezing), c: pseudo onset of ice melting, m: ice melting at maximum slope, p: peak of ice melting, e: end of ice melting

complex food systems, it was difficult to find a clear glass transition with thermal analysis since other changes interfere in the complexity of the structural glass. Glass transition was difficult to trace in foods with high amount of insoluble proteins and low amount of sugars, in the case of partial crystalline or other forms of ordering (as exist in native proteins), in foods exhibiting very low specific heat change at the glass transition and in cases of low plasticization effects of water on the protein (Rahman and Al Saidi, 2010). In addition, the formation of tightly packed aggregates reduced molecular mobility into lower nanometer range (i.e. instantaneous process tends to be very slow dynamic), which could be beyond the 20-300 nm range structural mobility determined by DSC (Rahman, 2010). In this case NMR or other spectroscopic techniques could be useful within 1-2 nm range. Green *et al.* (1994) concluded from their results that proteins indeed may be classed among

Table 3. Thermal characteristics of samples containing freezable water

X_w (g/g sample)	T_g''' (°C)	T_m' (°C)	ΔC_p (J/kg K)	T_a (°C)	T_m (°C)	T_p (°C)	ΔH (kJ/kg)
0.95	-16.2	-5.9	1695	-1.8	-0.3	2.9	199
0.90	-21.4	-8.9	1895	-3.3	-2.0	1.2	220
0.85	-23.5	-12.3	1756	-5.3	-4.6	-1.0	182
0.80	-24.1	-15.3	1169	-6.3	-5.5	-1.9	154
0.75	-31.4	-18.5	1227	-8.2	-6.7	-3.5	130
0.70	-36.3	-17.4	1853	-7.7	-4.0	-1.7	139
0.65	-37.8	-22.9	1608	-10.1	-8.5	-5.1	114
0.60	-36.9	-22.0	1730	-10.7	-8.2	-4.4	93
0.50	-34.4	-28.4	902	-15.2	-12.3	-8.8	70
0.40	-32.0	-27.0	933	-16.0	-14.9	-9.6	45
0.40[a]	-35.0 (4.0)	-30.0 (-2.0)	301 (78)	-23.5 (1.8)	-21.6 (1.4)	-14.6 (0.8)	35 (5)
0.30	-34.0	-29.3	60	-27.6	-24.6	-17.6	17

[a]Row indicates data are generated with 30 min annealing at T_m'-1
X_w :characteristic solid content.
T_g''' (°C): maximal-freeze concentration condition.
T_m' (°C): characteristic temperature of end point of freezing.
ΔC_p: Change in specific heat at the transition (kJ/kg K).
T_m (°C): melting temperature
ΔH (kJ/kg): enthalpy of melting.

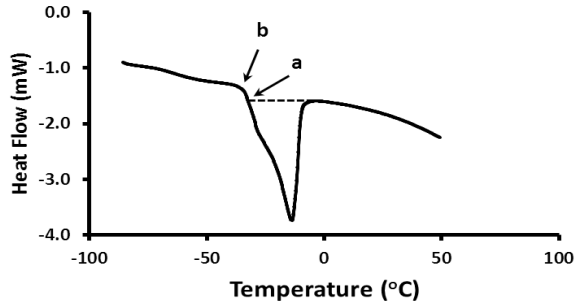

Figure 5. DSC thermogram of *A. bisporous* annealed at T_m'''-1 for 30 min annealing, a: (T_m')a (°C): apparent maximal-freeze concentration condition), b: (T_g''')a (°C): apparent maximal-freeze concentration condition)

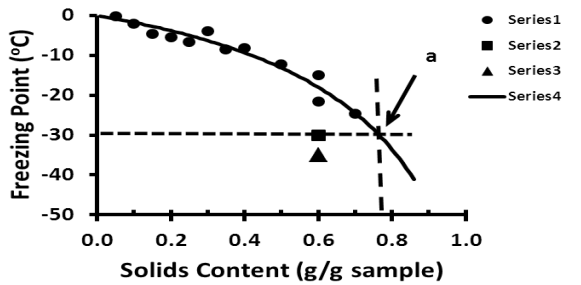

Figure 6. Freezing point of *A. bisporous* as a function of solids mass fraction (wet basis)

glass-forming systems, but due to their special structural features and to the disposition of the bound water, great departures from the thermo or rheological simplicity was observed.

The equilibrated samples containing moisture contents 0.167 and 0.295 g/100 g sample showed a trace of glass transition by a shift in the thermogram line as shown in the Figure 3 (marked as A). Table 2 shows the glass transition and specific heat change at the glass transition. As expected, glass transition decreased with the increase in moisture content since

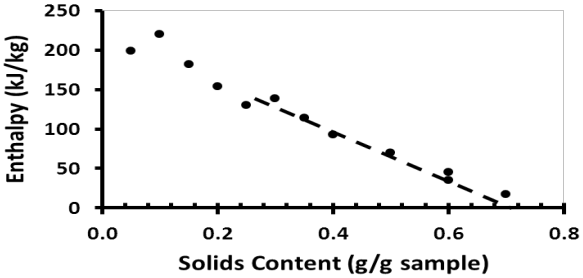

Figure 7. Plot of ice melting enthalpy as a function of water content

water acts as a plasticizer. Haiying *et al.* (2007) observed that the glass transition of fresh mushroom changed as a function of cooling rate. They reported that the partial glass transition temperatures of mushroom samples decreased when the cooling rate increased from 4.0 to 10.0°C/min, but when the cooling rates increased from 1.0 to 4.0°C/min, the trends were not significant. They also reported that the glass transition temperatures of fresh mushroom (moisture content: 0.887 g/g sample) measured at 1, 4 or 10°C/min were -63.1, -59 and -76.1°C, respectively.

Thermal transitions of freeze-dried mushroom containing freezable water

Figure 4 shows a typical thermogram of the sample with freezable water. This figure shows an endothermic peak due to the melting of ice during heating cycle and a shift just before the melting endotherm which was characterized as apparent *Tm'* and T_g'''. Haiying *et al.* (2007) measured the apparent T_m' as -21.4 ± 1.3°C of fresh mushroom (moisture content: 0.887 g/g sample) by cooling curve method, which is comparable with the apparent T_g''' measured by DSC method for the sample at moisture content 0.900 g/g sample (Table 3). Table 3 presents the freezing point, enthalpy at the ice melting, and actual maximal-freeze-concentration condition (T_m' and T_g'''). The actual $(T_m')_n$ and $(T_g''')_n$ were determined from sample having moisture content 0.400 g/100 g sample as -30 ± 2°C and -35 ± 4°C, respectively (Figure 5 and Table 2).

As expected, Figure 6 shows that the freezing point decreased with the increase of solids content in freeze dried mushroom. The model parameters E and B of equation 3 were estimated as 0.157 and -0.159 g/g dry solids ($p < 0.001$). The ultimate maximal-freeze-concentration, X_s' was determined from the intersection of the horizontal line passing through the actual, $(T_m')_n$ and extended-freezing curve (i.e. line of freezing point and solids content) developed by empirical model of freezing point data based on the Chen (1986) model. The value of X_s' was found as 0.782 g/g sample (Figure 6 as marked a). Thus,

the un-freezable water content of mushroom can be estimated as 0.218 g/g sample (i.e. X_{uw}=1- X_s').

The enthalpy change during melting of ice in the sample as a function of water content is shown in Figure 7. The un-freezable water content was estimated from drawing a line passing through the points at medium to low moisture contents. The line extending to enthalpy zero can be used to estimate the un-freezable water content as X_{uw} equal to 0.30 g/g sample. This method usually provides higher values (Rahman *et al.*, 2010). The errors could be due to two reasons: (i) enthalpy of pure water (heat of fusion) is a function of temperature, (ii) heat represented by the area under the curve is a combination of heat of fusion of ice plus sensible heat taken by freshly melted water. The estimation of un-freezable water is most acceptable and accurate when actual $(T_m')_n$ in the state diagram is used to determine the intersection point of the horizontal line passing through $(T_m')_n$ and the extended freezing curve following the same curvature (Rahman, 2004; Rahman *et al*., 2005).

Conclusion

The present study investigated various parameters related to the thermal Characteristics of *A. bisporus* Mushroom. The glass transition of *A. bisporus* containing un-freezable water was studied in order to determine the stability of dry mushroom during its storage, whereas T_m' to determine the stability for the frozen *A. bisporus*. It was not possible to trace glass transition in samples containing low water, which indicated the structural complexity of mushroom in terms of thermal behavior. However, samples containing higher water content exhibited glass transition. Maximal-freeze-concentrated solids was found as 0.782 g/g sample with the characteristic temperature of end point of freezing (T_m') being -30 ^oC. The un-freezable water (i.e., reactive water) in mushrooms was observed as 0.218 g/g sample.

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References

Anderson, R. B. 1946. Modifications of the Brunauer, Emmett and Teller equation. Journal of the American Chemical Society 68: 686–691.

AOAC. 1995. Official methods of analysis. 16th edn. Washington, DC: Association of Official Analytical Chemists.

Barros. L., Baptista. P., Correia, D. M., Casal, C., Oliveira, B. and Ferreira, I. C. F. R. 2007. Fatty acid and sugar compositions and nutritional value of five wild edible mushrooms from Northeast Portugal. Food Chemistry 105: 140-145.

Brunauer, S., Emmett, P. F. and Teller, E. 1938. The absorption of gases in multi-molecular layers. Journal of the American Chemical Society 60: 309–319.

Chen, C. S. 1986. Effective molecular weight of aqueous solutions and liquid foods calculated from the freezing point depression. Journal of Food Science 51 (6): 1537–1553.

Colak, A., Kolcuoğlu, Y., Sesli, E. and Dalman, Ö. 2007. Biochemical Composition of Some Turkish Fungi. Asian Journal of Chemistry 19: 2193-2199.

Diez,V. A. and Alvarez, A. 2001. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. Food Chemistry 75: 417-422.

Emynur Shafekh, S., Mohd Adzim Khalili, R., Catherine, C. C. W., Siti Syakiroh, Z. A., Ummu Habibah, A., Norhayati, A. H., Nor Farhanah, M. Y., Noor Husna, Z., Siti Nafizah, M. B., Azlina, M., Noor Shahhida Sazura, A. R. and Ahmad Zubaidi, A. L. 2012. Total phenolic content and in vitro antioxidant activity of *Vigna sinensis*. International Food Research Journal 19 (4): 1393-1400.

Fabra, M. J., Talens, P., Moraga, G. and Martinez-Navarrete, N. 2009. Sorption isotherm and state diagram of grapefruit as a tool to improve product processing and stability. Journal of Food Engineering 93 (1): 52–58.

Green, J. L., Fan, J. and Angell, C. A. 1994. The protein–glass analogy: some insights from homopeptide comparisons. Journal of Physical Chemistry 98: 13780–13790.

Guizani, N. and Sablani, S. S. 2007. 'Guest Editorial'. International Journal of Food Properties 10: 199.

Guizani, N., Al-Saidi, G. S., Rahman, M. S., Bornaz, S. and Al-Alawi, A. A. 2010. State diagram of dates: glass transition, freezing curve and maximal-freeze-concentration condition. Journal of Food Engineering 99 (1): 92-97.

Guizani, N., Al-Shoukri, A. O., Mothershaw, A. and Rahman, M. S. 2008. Effects of salting and drying on shark meat quality characteristics. Drying Technology 26 (6): 705–713.

Haiying, W., Shaozhi, Z. and Guangming, C. 2007. Experimental study on the freezing charcateristics of four kinds of vegetables. LWT-Food science and Technology 40: 1112-1116.

Hung, P. V. and Duy, T. L. 2012. Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants. International Food Research Journal 19 (1): 327-332.

Kalmiş, E., Yildiz, H., Ergonul. B., Kalyoncu, F. and Solak, M. H. 2011. Chemical composition and nutritional value of a wild edible ectomycorrhizal mushroom, *Tricholoma anatolicum*. Turkish Journal of Biology 35: 627-633.

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

Kim, D. O., Jeong, S. W. and Lee, C. Y. 2003. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. Food Chemistry 81: 321–326.

Ko, J. W., Lee, W. Y., Lee, J. H. and Ha, Y. S. 1999. Absorption characteristics of dried shiitake mushroom powder using different drying method. Korean Journal of Food Science and Technology 32: 12–137.

Kumari, D., Reddy, M. S. and Upadhyay, R. C. 2011. Nutritional composition and antioxidant activities of 18 different wild Cantharellus mushrooms of northwestern Himalayas. Food Science and Technology International 17 (6): 557-567.

Labuza, T. P. 1968. Sorption phenomena in foods. Journal of Food Technology 22 (3): 15–24.

Manzi, P., Aguzzi, A. and Pizzoferrato, L. 2001. Nutritional value of mushrooms widely consumed in Italy. Food Chemistry 73: 321-325.

Mau, J. L., Lin, H. C. and Song, S. F. 2002. Antioxidant properties of several specialty mushrooms. Food Research International 35: 519–526.

Mitchell, J. R. 1998. Water and food macromolecules. In Hill, S.E., Ledward, D.A. and. Mitchell, J. R. (Eds.). Functional Properties of Food Macromolecules, p. 50–76. Gaithersburg: Aspen Publishers.

Palacios, I., Lozano, M., Moro, C., D'Arrigo, M., Rostagno, M. A., Martínez, J. A., García-Lafuente, A., Guillamón, E. and Villares, A. 2011. Antioxidant properties of phenolic compounds occurring in edible mushrooms. Food Chemistry 128: 674–678.

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

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. 2009. Food Stability beyond water activity and glass transition: Macro-micro region concept in the state diagram. International Journal of Food Properties 12 (4): 726-740.

Rahman, M. S. 2010. Food stability determination by macro-micro region concept in the state diagram and by defining a critical temperature. Journal of Food Engineering 99 (4): 402-416.

Rahman, M. S. and Al-Saidi, G. 2010. Thermal relaxation of gelatin and date flesh measured by isothermal condition in differential scanning calorimetry (DSC) and its relation to the structural and mechanical glass transition. International Journal of Food Properties 13 (4): 931-944.

Rahman, M. S., Al-saidi, G., Guizani, N. and Abdullah, A. 2010. Development of state diagram of bovine gelatin by measuring thermal characteristics using differential scanning calorimetry (DSC) and cooling curve method. Thermochimica Acta 509: 111-119.

Rahman, M. S., Kasapis, S., Guizani, N. and Al-Amri, O. 2003. State diagram of tuna meat: freezing curve and glass transition. Journal of Food Engineering 57 (4): 321–326.

Rahman, M. S., Sablani, S. S., Al-Habsi, N., Al-Maskri, S. 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): E135–E141.

Roos, Y. H. 1987 Effect of moisture on the thermal behavior of strawberries studies using differential scanning calorimetry. Journal of Food Science 52: 146-149.

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

Sanmee, R., Dell, B., Lumyong, P., Izumori, K. and Lumyong, S. 2003. Nutritive value of popular wild edible mushrooms from northern Thailand. Food Chemistry 84: 527-532.

SAS. 2001. Statistical Analysis Systems, STATR Software: Changes and Enhancements (Release 6.02). SAS Technical Report, Cary, North Carolina: SAS Institute Inc.

Seiiedlou, S., Ghasemzadeh, H. R., Hamdami, N., Talati, F. and Moghaddam, M. 2010. Convective drying of apple: Mathematical modeling and determination of some quality parameters. International Journal of Agriculture and Biology 12 (2): 171–178.

Singleton V. L. and Rossi J. A. 1965. Colorimetry of total phenolics with phosphomolybdicphosphotungstic acid reagents. American Journal of Enology and Viticulture 16: 144-158.

Spiess, W. E. L. and Wolf, W. 1987. Critical evaluation of methods to determine moisture sorption isotherms. In Rockland, L.B. and Beuchat, L.R. (Eds.). Water Activity: Theory and Applications to Foods, p. 215–234. New York: Marcel Dekker.

Tsami, E., Krokida, M. K. and Drouzas, A. E. 1999. Effect of drying method on the sorption characteristics of model fruit powders. Journal of Food Engineering 38: 381–392.

Yildiz, A., Yeşil, Ö. F. and Yavuz, Ö. 2005. Organic elements and protein in some macrofungi of south east Anatolia in Turkey. Food Chemistry 89: 605-609.