ELSEVIER

Thermochimica Acta 349 (2000) 79–88

thermochimica acta

www.elsevier.com/locate/tca

Heat capacity of birch determined by calorimetry: implications for the state of water in plants

Henrik Hedlund[a,*], Peter Johansson[b]

[a]*Department of Agricultural Biosystems and Technologies, Swedish University of Agricultural Sciences, P.O. Box 44, S-230 53, Alnarp, Sweden*

[b]*Thermochemistry Chemical Centre, University of Lund, P.O. Box 124, S-221 00, Lund, Sweden*

Received 21 December 1998; received in revised form 10 June 1999; accepted 12 July 1999

Abstract

Heat capacity measurements have been performed on birch plants (*Betula pendula* Roth.) by use of a drop-Cp-microcalorimeter. Influence of hydration level on heat capacity was investigated. It was found that heat capacity decreased in a linear fashion with decreasing water content until a point of discontinuity was reached at a water fraction of 0.2 g g^{-1} (grams of water per gram fresh biomass). A linear regression of the region above 0.2 g g^{-1} gave a slope of 2.49 J K^{-1} g^{-1} and an intercept of 1.72 J K^{-1} g^{-1}. It was concluded that a rough division of the water in the biomass into free and perturbed water is reflected in the heat capacity of the system and that the heat capacity in the region below 0.2 g g^{-1} could be divided into three discrete subregions. These subregions are assumed to reflect the perturbed water state in the tissue at different levels of hydration.

Further, the temperature influence on heat capacity was studied in the range between 14 and 37°C. The water fraction in these experiments was 0.75±0.1 g g^{-1}. No significant difference in heat capacity values was observed. It was concluded that this result reflects the dominant influence of free water on heat capacity at this level of water content.

Keywords: Birch; Calorimetry; Heat capacity; Temperature; Water

1. Introduction

Water in biological tissues has been studied with a range of different techniques, e.g. radioactive labeling, NMR, phosphorescence, DSC (differential scanning calorimetry). Many of these studies suggest that the properties of water in biological systems are different from that of pure water or water in a salt solution. This water has been described as structurally modified water, bound water or perturbed water, in contrast to bulk water, pure water or free water [1,2,8]. For consistency in this text we will use the terms perturbed water and free water.

Measurements of the diffusion coefficient of water points to a considerably lower value in cell cytoplasm than the self diffusion coefficient of free water [3,4]. Other studies using labeled inert molecules of different sizes, which were allowed to penetrate the plasma membrane into the cytoplasm of animal (3T3) cells,

* Corresponding author. Tel.: +46-40-415477; fax: +46-40-465743.
E-mail address: henrik.hedlund@jbt.slu.se (H. Hedlund)

0040-6031/00/$ – see front matter
PII: S0040-6031(99)00499-2

also show a lower value of the diffusion coefficient than would be expected for free water [5].

A biophysical explanation of the difference in properties of water can be found by studying the physical interactions between water molecules and cellular substances (e.g. solutes, macro-molecules) or water molecules and structures (e.g. membranes). Electrostatic interactions are crucial for the stabilization of macromolecular structures in their native form. Charged or polar groups in these structures influence adjacent water dipoles, which cause a decrease in the mobility of the water molecules [6]. This decrease is mainly due to hydrogen bond formation and leads to changes in physical properties like compressibility and partial volume [7] and in thermodynamic properties like entropy and heat [8].

Non-polar groups in macromolecular structures also have a structurally stabilizing effect on water, through hydrophobic interactions [1,2,9].

The interaction between water and macromolecules has been described theoretically in a model by statistical mechanics [10]. This model has been shown to give good predictions of the process of protein hydration.

Thermodynamic investigations have also contributed to the present view of the nature of perturbed water in biological systems. Studies of melting transitions with differential scanning calorimetry (DSC) indicate a classification of perturbed and free water, described in terms of non-freezable and freezable water [11,12].

Heat capacity measurements have provided valuable insight into the nature of water associated with different macromolecules, because of the large changes in heat capacity as the water binds to a surface. For instance, in [13] the relations between heat capacities of dry protein and diluted protein solutions were investigated for a water–lysozyme system. Heat capacity determination of other mixed systems have also been performed, e.g. for a water–starch system [14] and for water–cellulose and water–oligosaccharides systems [15].

Different levels of hydration strongly affect physiological activities of seeds [16]. It is concluded that few reactions occur at low hydration, but that the metabolism of the system becomes increasingly facilitated with increasing hydration. Germination of the seed was not observed until the seeds had attained moisture contents beyond full hydration. Therefore, calorimetric techniques (e.g. DSC) have been used to study the state of water in hydrating seeds of pea and soybean [17]. The heat capacity as a function of water content in seeds has a complex relationship in regions with low hydration and a linear relationship in the fully hydrated region.

We propose that heat capacity measurements of plant tissues with different water contents will provide valuable insight into the nature of water in complex biological structures. Measurements of heat capacity has therefore been conducted for different plant parts (root, leaf and stem) of birch (*Betula pendula* Roth.) in a series of calorimetric experiments. The results from these experiments give a description of how the heat capacity depends on water content, growth stage and temperature.

This work can be viewed partly as a methodology work, since (as far as we know) it is the first time a living biological material has been measured with the used technique.

2. Material and methods

2.1. Growth experiment

Birch seeds were germinated in petri dishes for 10–14 days and then placed in an aeroponic growth unit (Biotronic AB, Uppsala, Sweden), where nutrient solution was sprayed on the roots and recirculated in a closed system. The growth technique is described in detail in [18,19]. Stock solutions were composed and prepared according to [19], giving a solution that contains all essential nutrients in balanced proportions. Non-limiting availability to all nutrients was secured by regular titrations.

The first stage of growth (pregrowth) lasted approximately 2 weeks, to exhaust seed resources and to produce seedlings equal in size and composition. In the second stage, the main experiment, plants were grown under steady-state conditions and repeated harvests were made to measure biomass and other plant quantities.

The plants were grown in a controlled environmental room at the Biotron in Alnarp, Sweden. During the pregrowth, air and root temperature were 20°C and the relative humidity was 70%. Photon flux density (PFD) was 250±20 mmol m^{-2} s^{-1} at the top of the plants,

which corresponds to a daily quantum flux of 17–20 mol m^{-2} per day. During the main experiment photon flux density was raised to 350±20 mmol m^{-2} s^{-1} (28–32 mol m^{-2} per day), which was the saturating light level for growth of birch [20].

The growth method provides controlled uptake of nutrients, water and carbon, which yields plants with a stable physiological state. This means that the nutrient and carbon concentrations and the water fraction in the biomass are constant during the growth experiment. Samples of seedlings used for calorimetric measurements of heat capacities, therefore, have specific and accurate proportions of different elements in the biomass.

2.2. Sample preparation

The samples for calorimetric determination of heat capacities were prepared both at the end of pregrowth and at five occasions during the main experiment. The fresh biomass was measured for each individual plant. After weighing, the plants were immediately put in test tubes, filled with nutrient solution from the growth unit, and placed in a box for transportation to the Division of Thermochemistry, Lund University, Sweden. This procedure was repeated in different series of experiments. One series was performed on samples with varied water contents at a temperature of 20°C. Another series was performed at different temperatures, i.e. at 14, 20, 25, 31 and 37°C. A third series was performed with varied sample sizes, i.e. for different growth stages of the birch plants. Before charging the calorimeter the plant was divided into roots, stems and leaves.

In order to study the dependence of water content on heat capacity, in some experiments sample preparation included a partial drying. Between measurements, the plunger of the measuring container was opened and the ampoule was flushed with dry nitrogen until a desired water content was attained (determined by repeated weighing). The partial drying was not performed for experiments studying the effect of temperature and growth stage.

2.3. Determination of biomass and water content

The mass of the sample was determined prior to measurement by weighing the ampoule before and after it was charged. After the calorimetric measurement the tissue was placed in an oven at 60°C for 4–6 h, followed by half an hour in 110°C. This procedure was sufficient to attain a constant weight of the sample. The water content was estimated by subtracting the mass of the fresh sample from that of the dry sample.

2.4. Heat capacity measurements

The heat capacity of the birch samples was determined by means of a drop-calorimeter, which mainly consists of a furnace and a receiving calorimeter. The temperature of the furnace is kept constant to better than ±0.0002 K by means of a Eurotherm proportional controller (Eurotherm, Worthing, Sussex, England). The calorimeter is positioned in a water thermostat, in which the temperature is kept constant to about ±0.0005 K using a Eurotherm proportional controller. The calorimeter is intended for precise measurements on small solid or liquid samples, weighing less than 1 g.

During measurement, the sample was contained in a 1 ml cylindrical container made of stainless steel. The mass of the sample varied from 5 to 20 mg at the initial stage to 100–150 mg for the last harvested plants. The container was sealed with a plunger fitted with an *o*-ring, keeping it water- and air-tight to prevent water losses. The container was allowed to equilibrate in the furnace for 25 min after which it was dropped into the receiving calorimetric unit. The time for the calorimeter to reach equilibrium after a drop was approximately 30 min. For all experiments the temperature difference between the furnace and the calorimeter, $\Delta\theta$, was about 9.5 K. The temperature difference between the furnace and the calorimeter can be measured with an estimated accuracy of 0.001 K. An initial temperature, θ_i, was measured immediately before the container was dropped. A final temperature, θ_f, was measured at the end of each run. The mean heat capacity in the temperature range from θ_i to θ_f (or the heat capacity for the midpoint temperature between θ_i and θ_f) is considered to be equal to the transferred heat divided by the temperature difference $\theta_i - \theta_f$. The temperature difference is assumed to be small enough to give an adequate heat capacity value for the midpoint temperature.

Calibration of the instrument was made with a few droplets of water of Millipore quality (ca. 100 mg) in

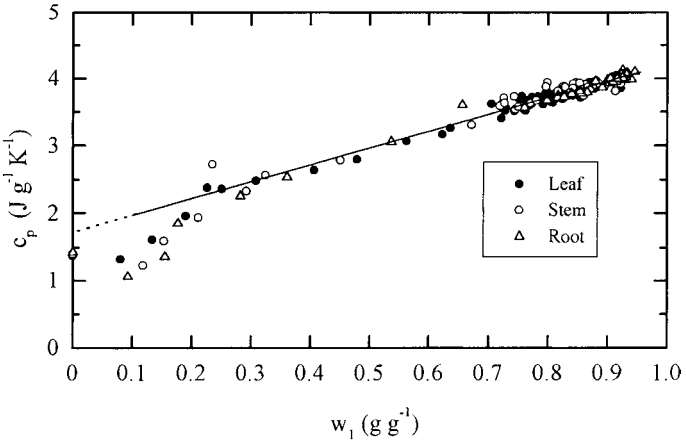

Fig. 1. Specific heat capacity (c_p) as a function of water fraction (w_1) for leaf, stem and root of birch, respectively. The solid line represents a linear regression of the total dataset.

the sample container. The baseline stability of the calorimeter was approximately 0±4 mJ. Detailed measuring procedure and design of the instrument is described elsewhere [21].

Each measurement was repeated 2–3 times, in order to specify the accuracy in the measured heat capacity values. The accuracy was in the order of 0.1% in the temperature range for the different experiments.

3. Results

3.1. Water content

Heat capacity as a function of water content, expressed as a fraction of fresh biomass (w_1), is shown in Fig. 1. A linear equation ($y=kx+m$) was fitted to heat capacity data above $w_1=0.2$ g g^{-1}, by means of least square regression. This procedure was performed separately on datasets for different plant parts, but also on the total dataset for all plant parts. The results from the regressions are given in Table 1. Linear relationships were highly significant for all datasets. The slope k is 2.49 J g^{-1} K^{-1} and the intercept m was 1.72 J g^{-1} K^{-1} for the total dataset. The corresponding values for the individual datasets of leaves and stems were, within error limits, the same as for the total dataset. For roots, there was a small but significant deviation in the slope (k=2.60 J g^{-1} K^{-1}) and in the intercept (m=1.62 J g^{-1} K^{-1}). In the range below $w_1=0.2$ g g^{-1}, the heat capacity showed no clear correlation with water content.

Plants that were not partially dried had a water fraction in the range $w_1=0.70$–0.95 g g^{-1}. This range represents water contents normally found in plants at physiological conditions.

3.2. Temperature and growth stage

In order to determine the dependence of growth stage and temperature on plant heat capacity, the

Table 1
Specific heat capacity (c_p) as a function of water fraction (w_1) in the fully hydrated region[a]

Dataset	Samples	w_1 (g g^{-1})	c_p (J g^{-1} K^{-1})	k (J g^{-1} K^{-1})	m (J g^{-1} K^{-1})	R
Leaves	63	0.23–0.92	2.37–3.95	2.45±0.064	1.75±0.049	0.980
Stems	53	0.21–0.92	1.94–3.97	2.48±0.090	1.75±0.072	0.968
Roots	54	0.28–0.95	2.26–4.10	2.60±0.060	1.62±0.052	0.986
Total	170	0.21–0.95	1.94–4.10	2.49±0.041	1.72±0.034	0.978

[a] The results from the regression analysis describe the slope (k), the intercept (m) and the correlation coefficient (R) for this function, based on data for leaves, stems, roots and for the total dataset, in the fully hydrated region above $w_1=0.2$ g g^{-1}.

measurements must be compensated for the influence of differences in water content between samples. This was done by shifting the data in parallel to a common average water fraction (w_1=0.75 g g^{-1}), with the slope obtained from the regression line for the total dataset. Fig. 2 shows the dependence of temperature (A) and growth stage (B) on the heat capacity for leaves, stems and roots.

A test for differences in heat capacity between different temperatures and different growth stages was performed with a single factor variance analysis. The statistical model used was

$$y_{ij} = \mu + \tau_i + \varepsilon_{ij} = \mu_i + \varepsilon_{ij}, \quad i = 1, 2, \ldots, N;\ j = 1, 2, \ldots, M \tag{1}$$

where the index i refers to a treatment and the index j refers to an observation within a certain treatment among M observations. μ is the overall mean value in a specific dataset, τ_i is the effect of treatment i in a specific dataset among N treatments, ε_{ij} is the random error component and μ_i is the mean value for a specific treatment i. For reference, see for instance [22]. In our case, the overall mean value μ is the mean heat capacity for a given dataset, such as for the dataset of leaves, and the treatment effect τ_i is either different temperatures or different growth stages. The hypothesis (H_0) that the mean values of different treatments is equal

$$H_0: \quad \mu_1 = \mu_2 = \cdots = \mu_i = \cdots = \mu_N \tag{2}$$

was tested against the counter hypothesis (H_1) that the mean values differ from each other

$$H_1: \quad \mu_m \neq \mu_n \tag{3}$$

for at least one pair m, n. The mean values were estimated from the different datasets. The test was made for separate datasets of leaves, stems and roots, respectively, and for the total dataset. No significant differences were found in heat capacity with respect to temperature or growth stage (H_0 could not be rejected at the 99% confidence level).

4. Discussion

4.1. Fully hydrated region

The curve in Fig. 1 shows linearity in the region where the water mass fraction w_1 is larger than approximately 0.2 g g^{-1}. At w_1=0.2 g g^{-1} we suggest that the biomass was fully hydrated. Additional water in the biomass will then dilute the system with free water. This is in agreement with findings reported by Yang and Rupley [13] where the specific heat capacity of a water–lysozyme system shows linearity for water fractions above w_1=0.27 g g^{-1}. Noel and Ring [14] reported a linear region that begins approximately at a water fraction of w_1=0.2 g g^{-1} for a mixture of water and starch.

Therefore, we suggest a model for the linear region, where the plant tissue can be divided into free water and fully hydrated biomass (dry biomass and perturbed water). Quantitatively, this means an additivity in the total heat capacity. That is, the total heat capacity of the biological system can be approximated by the sum of heat capacities for the free water and the fully hydrated biomass

$$C_p = C_{p,FW} + C_{p,FHB} \tag{4}$$

where C_p is the total heat capacity, $C_{p,FW}$ is the heat capacity for the free water and $C_{p,FHB}$ is the heat capacity for the fully hydrated biomass. As a result of this additivity, the regression line can be identified with an equation where the total heat capacity is a weighed sum between the specific heat capacities of free water and fully hydrated biomass

$$C_p = mc_p = m(w_1 - w_1^*)c_{p,FW} + m[1 - (w_1 - w_1^*)]c_{p,FHB} \tag{5}$$

where m is the total biomass, c_p is the specific heat capacity for the total biomass, w_1 is the water mass fraction, w_1^* is the water mass fraction at full hydration, $c_{p,FW}$ is the specific heat capacity for free water and $c_{p,FHB}$ is the specific heat capacity for fully hydrated biomass at $w_1 = w_1^*$. After rearranging Eq. (5) can be written as

$$c_p = w_1(c_{p,FW} - c_{p,FHB}) + c_{p,FHB} - w_1^*(c_{p,FW} - c_{p,FHB}) = kw_1 + m \tag{6}$$

This equation is identified with the regression model, where the slope k is the difference between the specific heat capacities of free water and fully hydrated biomass. The intercept m is considered to be an ideal specific heat capacity of the dry biomass, where the linear region is extrapolated to w_1=0.

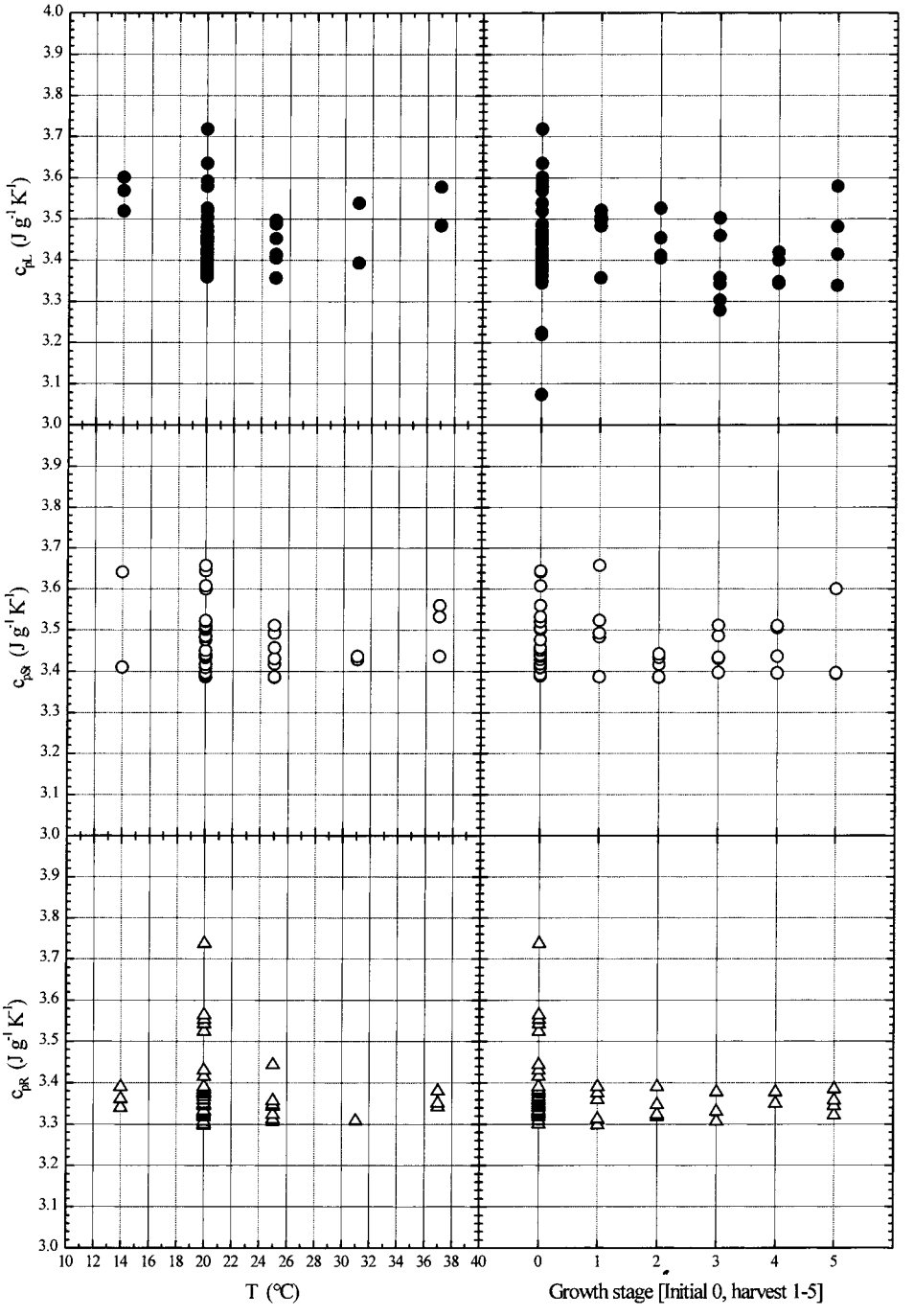

Fig. 2. Specific heat capacity as a function of temperature (A) and growth stage (B) for leaf (c_{pL}), stem (c_{pSt}) and root (c_{pR}) of birch, respectively.

The values for k and m agree well with results obtained on simpler systems, see Table 2 (region IV). In all the systems, birch, water–lysozyme [13] and water–starch [14], the regression parameters (k and m) add up to 4.2 J g^{-1} K^{-1}, corresponding to a system of pure water (Eq. (6)).

4.2. Partly hydrated region

The region below the fully hydrated region represents a state of the water–solid system where the water molecules are bound or perturbed relative to that of the free water molecules. These bonds represent different kinds of interactions, the nature of which depends on the hydration level of the sample. The partly hydrated region can be divided into a number of subregions. In the present discussion, four regions will be characterized according to Noel and Ring [13] for the water–lysozyme system. In our case, the apparent specific heat capacity for the dry biomass in the water–solid system is defined as

$$\phi c_{p2} = \frac{c_p - w_1 c_{p1}^0}{w_2} \quad (7)$$

where c_{p1}^0 is the specific heat capacity for pure water and w_2 is the fraction of dry biomass. In Fig. 3 ϕc_{p2} is plotted against the grams of water per gram dry biomass, defined as

$$h = \frac{w_1}{w_2} \quad (8)$$

Different subregions of hydration is indicated with Roman numbers (I–IV). The water–protein structure at different hydration levels is described by Rupley et al. [8], which can serve as a model structure for the discussion below. However, the water–tissue structure of birch may differ significantly from the model structure, since protein is a minor constituent in the biomass of birch.

Rupley et al. [8] proposed that the first region (I) in the model structure represents a state where the water molecules are most tightly bound, mainly to charged groups on the protein (see also Collins [6]). Initially, ϕc_{p2} for the protein decreases to a minimum after which the second region begins. In a narrow range of h near the minimum value there is a structural change of the hydrated water molecules, due to a transition from a disordered to an ordered state. Such pattern is indicated in subregion I in Fig. 3.

The second region described by Rupley et al. [8] is characterized by a linear increase in ϕc_{p2} with an increase in hydration, that is greater than that for free water. It was suggested that this originated from temperature-dependent water cluster rearrangements [8]. These clusters are principally bound to charged and polar sites on protein surfaces. Such response on increased hydration is indicated in subregion II in Fig. 3.

The third region in [8] begins with a transition region for ϕc_{p2}. The polar sites are nearly saturated and condensation of water takes place over the remaining unfilled portions of the surface. The inter-

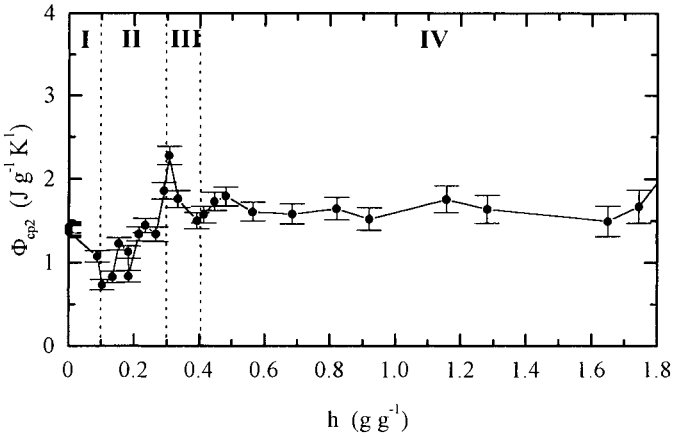

Fig. 3. Apparent, specific heat capacity for dry biomass ($\phi c_{p,2}$) as a function of water fraction on dry biomass basis (h), plotted for the total dataset in the range of h between 0 and 1.8 g g^{-1}.

Table 2
Summary of calorimetric properties for different water–solid systems in different subregions of hydration, described in accordance with Rupley et al. [8][a]

System	Region										Temperature (°C)	Reference
	I			II			III		IV			
	c^0_{p2} (J g^{-1} K^{-1})	h (g g^{-1})	$\phi c_{p2,min}$ (J g^{-1} K^{-1})	k=dc_p/dw_1 (J g^{-1} K^{-1})	h (g g^{-1})	$\phi c_{p2,max}$ (J g^{-1} K^{-1})	h (g g^{-1})	k=dc_p/dw_1 (J g^{-1}K^{-1})	h (g g^{-1})	m (J g^{-1} K^{-1})		
Water–lysozyme	1.26	0–0.07	1.23	4.5	0.07–0.26	1.57	0.26–0.38	2.7	0.38	1.5	25	[13]
Water–starch	1.16	–	–	4.5	–	–	–	2.5	0.37	1.7	25	[14]
Water–cellulose	1.24	0–0.08	1.3	4.1	–	–	–	–	–	–	47	[15]
Pea seeds	1.44	0–0.14	0.78	6.36	0.14–0.34	1.47	0.34–0.46	–	–	–	–60	[16]
Soybean seeds	1.42	0–0.09	1.04	6.10	0.09–0.27	1.72	0.33–0.38	–	–	–	−60	[16]
Birch	1.38	0–0.1	0.75	6.44	0.1–0.3	2.47	0.3–0.4	2.49	0.4	1.72	25	

[a] The level of hydration is expressed as grams of water per gram dry biomass (h). c^0_{p2} is the heat capacity for the dry substance in the system, $\phi c_{p2,min}$ is the minimum, apparent specific heat capacity for the dry substance in the system, k is the slope from a linear regression of specific heat capacity (c_p) as a function of water fraction (w_1), $\phi c_{p2,max}$ is the maximum, apparent specific heat capacity for the dry substance in the system and m is the apparent specific heat capacity for the dry substance at full hydration (identical with intercept m in the linear model described in Eq. (6)).

actions between water molecules and protein surfaces are primarily built up by non-polar, hydrophobic bonds. There is a slight decrease in heat capacity at the transition, probably due to a lowering of the degrees of freedom [16]. Such a decrease is indicated in subregion III in Fig. 3.

In the fourth region described by Rupley et al. [8], ϕc_{p2} is considered to have a constant value. A monolayer of water molecules now covers the protein surface and the additional perturbation of adding more water will be dependent on weak interactions. The mobility of the additional water will be close to free water, so the addition will merely dilute the water–solid system. This region is therefore equivalent to the fully hydrated region described earlier. A constancy in ϕc_{p2} is indicated in subregion IV in Fig. 3.

An overview of the different subregions for birch, water–lysozyme, water–starch, water–cellulose, pea seed and soybean seed is given in Table 2. All experiments are not quantitatively comparable to our data because the determination of ϕc_{p2} was performed at different temperatures. However, experimental temperatures are identical for the experiments done on birch, water–lysozyme and water–starch.

Heat capacity values for the dry substance of different systems lie approximately in the range c^0_{p2}= 1.2–1.4 J g^{-1} K^{-1}. Estimated values of c^0_{p2} for seeds of pea and soybean at −12.5°C were obtained from the lowest water fraction in Figs. 6 and 7 in [16], by extrapolating to zero water fraction with the same negative slope as for birch in region I (Fig. 3). c^0_{p2} for birch is somewhat higher than for the simpler systems but in close agreement with the estimations from the seed experiments.

The hydration levels for region I–IV are for birch roughly in the ranges h=0–0.1, 0.1–0.3, 0.3–0.4 and 0.4–1.0 g g^{-1}, respectively. This is in accordance with an estimation of corresponding ranges for the other systems, where a range identification has been possible.

The estimated extreme values for ϕc_{p2} at the transitions at the end of regions I and II, respectively, are comparable for birch and water–lysozyme. A comparison gives a significant difference for both $\phi c_{p2,min}$ and $\phi c_{p2,max}$ between those systems ($\phi c_{p2,min}$=0.75 J g^{-1} K^{-1} for birch and $\phi c_{p2,min}$=1.23 J g^{-1} K^{-1} for water–lysozyme; $\phi c_{p2,max}$=2.28 J g^{-1} K^{-1} for birch and $\phi c_{p2,max}$=1.57 J g^{-1} K^{-1} for water–lysozyme).

A linear regression of c_p as a function of w_1 corresponding to region II gives a value for the slope of 6.44 J g^{-1} K^{-1} for birch. This value is in accordance with estimations for pea seeds and soybean seeds, but differs from values estimated for the simpler systems.

A decreasing value of ϕc_{p2} in region III with an increase in h is noticed for both birch and water–lysozyme. In region IV ϕc_{p2} approaches an asymptotic value of approximately 1.7 J g^{-1} K^{-1} for birch and 1.5 J g^{-1} K^{-1} for water–lysozyme, which is identified with the apparent heat capacity for the dry substance at full hydration. ϕc_{p2} is here identical with the intercept m for the linear model described in Eq. (6).

Structural information about the change of water state can not be gained from heat capacity measurements alone, but the information can be used to support models involving structural parameters. One empirical model for the change in ϕc_{p2} with h is described in [13]. Such a phenomenological description can further be explained with a statistical mechanical model, showing how adsorbed molecules interact on a heterogeneous surface [10].

4.3. Dependence of temperature and growth stage

There are no significant differences in c_p for birch in the studied temperature range (14–37°C). The constancy in this thermodynamic property probably reflects the dominance of free water in the birch samples in this experiment series (w_1=0.70–0.95 g g^{-1}). The heat capacity of pure water in this range is almost constant.

The change of c_p with temperature for the dry biomass was not studied, but the magnitude of change can be indicated by the corresponding values for the water–starch system [14]. In the temperature range 7–70°C the specific heat capacity for dry starch increased linearly from 1.12 to 1.37 J g^{-1} K^{-1}. For water–starch mixtures with low water content (w_1=0.14 and 0.17 g g^{-1}, respectively) the increase of c_p was non-linear within the same range. The apparent heat capacity for water in those mixtures was approximately constant in the whole range and equal to 4.7 and 4.8 J g^{-1} K^{-1}, respectively.

The temperature dependence of c_p as a result of changing interactions between water and macromole-

cules has been further studied by Privalov et al. [23] and Makhatadze et al. [24].

The constancy of c_p for birch at different growth stages during the exponential growth can be explained with the stability of the physiological state, comprising of constant concentrations and proportions of nutrients, carbon and water. This is a result of the experimental control in the method of growing birch seedlings and the stability has been confirmed in a comprehensive set of experiments, see for instance [20].

Acknowledgements

The authors wish to thank professor Ingemar Wadsö and professor Göran Ågren for valuable comments on the manuscript.

References

[1] W. Drost-Hansen, in: H.D. Brown (Ed.), Chemistry of the Cell Interface, Academic Press, New York, 1971.
[2] I.D. Kuntz, W. Kauzmann, Advances in Protein Chemistry 28 (1974) 239.
[3] D.C. Chang, H.E. Rorschach, B.I. Nichols, C.F. Hazlewood, Annals New York Academy of Sciences 704 (1973) 434.
[4] K. Hansson-Mild, S. Lövtrup, Biochimica et Biophysica Acta 822 (1985) 155.
[5] K. Luby-Phelps, D.L. Taylor, F. Lanni, Journal of Cell Biology 102 (1986) 2015.
[6] K.D. Collins, Biophysical Journal 72 (1997) 65.
[7] T.V. Chalikian, A.P. Sarvazyan, K.J. Breslauer, Biophysical Chemistry 51 (1994) 89.
[8] J.A. Rupley, E. Gratton, G. Careri, Trends in Biochemical Sciences Vol. 8 (1983) 18.
[9] F. Franks, in: F. Franks (Ed.), Water: a Comprehensive Treatise, Plenum Press, New York, 1982, p. 215.
[10] T.L. Hill, Journal of Chemical Physics 17 (1949) 762.
[11] E.L. Andronikashvili, G.M. Mrevlishvili, Lèau et les Systèmes Biologiques 246 (1975) 275.
[12] A.D. Brown, J.M. Sturtevant, Journal of Membrane Biology 54 (1980) 21.
[13] P.H. Yang, J.A. Rupley, Biochemistry 18 (1979) 2654.
[14] T.R. Noel, S.G. Ring, Carbohydrate Research 227 (1992) 203.
[15] T. Hatakeyama, H. Hatakeyama, Cellulose and its derivatives: chemistry, biochemistry and applications, in: J.F. Kennedy (Ed.), Series in Organic Chemistry, Ellis Horwood, New York, 1985, p. 87.
[16] C.M. Vertucci, Biophysical Journal 58 (1990) 1463.
[17] C.W. Vertucci, Physiologia Plantarum 77 (1989) 172.
[18] T. Ingestad, A.-B. Lund, Physiologia Plantarum 45 (1979) 137.
[19] T. Ingestad, A.-B. Lund, Scandinavian Journal of Forest Research 1 (1986) 439.
[20] T. Ingestad, O. Hellgren, A.-B. Lund-Ingestad, Swedish University of Agricultural Sciences — Report, Vol. 75, 1995, p. 1.
[21] J. Suurkuusk, I. Wadsö, Journal of Chemical Thermodynamics 6 (1974) 667.
[22] D.C. Montgomery, Design and Analysis of Experiments, 3rd Edition, Wiley, New York, 1991, p. 53.
[23] P.L. Privalov, G.I. Makhatadze, Journal of Molecular Biology 224 (1992) 715.
[24] G.I. Makhatadze, M.M. Lopez, P.L. Privalov, Biophysical Chemistry 64 (1997) 93.