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Specific heat determination of plant barrier lipophilic components: biological implications

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Abstract

The specific heat of isolated plant cuticles and their corresponding cuticular waxes have been measured for the physiological temperature in the range of 273–318 K at regular intervals. C_p values ranged from 1.5 up to 4 J K^{-1} g^{-1} indicating a high cohesion, at the molecular level, of the molecular lipophilic components that constitute the plant cuticle. Second order phase transitions around 293 K, assigned to the cuticular matrix mainly constituted of the biopolyester cutin, have been detected and measured. Ecophysiological and physical implications of these thermodynamic data are discussed. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Plant cuticle; Wax; Specific heat; Second order phase transition

1. Introduction

Cuticular membranes or cuticles of higher plants are chemically heterogeneous in nature consisting mainly of a wax fraction, soluble in common organic solvents, and an insoluble lipophilic matrix, namely cutin, which constitutes the framework of the cuticle. The main function ascribed to plant cuticle is to minimise water loss. In addition, it limits the loss of substances from plant internal tissues and also protects the plant against physical, chemical and biological aggressions [1]. The cuticle forms an important barrier to the uptake of foliar pesticides which have to penetrate the cuticle in order to develop their physiological action in plant cells [2].

Plant cuticular material occurs in considerable amounts in both natural and agricultural plant communities: between 180 and 1500 kg per hectare. Weight of isolated cuticles range between 2000 μg cm^{-2} (fruit cuticles) and 450–800 μg cm^{-2} (leaf cuticles) of which 55–70% corresponds to the biopolymer cutin [3]. These data place cutin as the third most abundant plant polymer after the polysaccharide cellulose and the complex biopolymer lignin.

Cuticular waxes are complex mixtures of very long chain fatty acids, alcohols, aldehydes, esters and *n*-alkanes [4]. In some plants, compounds such as cyclic terpenoids and phenolics can also be present. These mixtures of non-polar compounds are deposited at regular intervals of time in the outer surface of leaves and fruits forming crystalline and amorphous regions, the mechanism of which is still one of the mysteries of cuticular wax production. The crystalline-amorphous structures of cuticular waxes are

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most likely to result from the physicochemical properties of the wax rather than as a result of a concrete transport process.

The cuticular matrix is mainly formed by the biopolymer cutin which is a high molecular weight polyester composed of various inter-esterified C_{16} and C_{18} hydroxyalkanoic acids [5]. Structural and physicochemical studies on cutin have been previously reported by our research group using Fourier transform infrared (FT-IR) spectroscopical studies [6,7] and X-ray diffraction analysis [8]. The results suggested the existence of an amorphous structure in the cuticular matrix with basal spacing around 0.45 nm of a repeat unit in the macromolecular structure of cutin. Additionally, solid state ^{13}C-nuclear magnetic resonance (NMR) studies of the polyester cutin provided valuable structural information on the intact biopolymer, identifying distinct polymer molecular domains [9].

Temperature-dependent changes in the properties of the plant cuticle and in the interactions between their different components (waxes, cutin and minor polar components as cellulose and phenols) are not well understood. Eckl and Gruler [10] carried out a calorimetric experiment with wet *Citrus aurantium* and *Hedera helix* leaf cuticles. They reported the existence of weak latent heat between 16 and 40°C. Schreiber and Schönherr [11], while calculating volume expansion coefficients, found that isolated cuticles of several species exhibited second order phase transitions in the temperature range of about 40–50°C. Recently, Luque and Heredia [12] have reported the occurrence of a glass transition temperature in isolated tomato fruit cutin at −47°C together with a weak secondary phase transition around 30°C.

It is well established that the specific heat or heat capacity, C_p, is the most sensitive thermodynamic indicator of structure [13]. The present work deals specifically with the determination of the heat capacity and its temperature variation of reconstituted cuticular waxes, isolated cuticles and cuticular matrices of several plant species. This study reflects for the first time a different level of our understanding of the macromolecular structure of the different components of the plant cuticle, with specific and concrete ecophysiological implications.

2. Material and methods

2.1. Cuticle isolation

Cuticular membranes were isolated from mature grape berries (*Vitis vinifera* L.), mature tomato fruits (*Lycopersicon esculentum* Mill.) and sour orange leaves (*C. aurantium* L.). Isolation was carried out in an aqueous solution of ammonium oxalate (1.6%, w/v), oxalic acid (0.3%, w/v) [8]. After 4–7 days the cuticles were separated from the outer wall of the epidermis. After isolation, the cuticles were extensively washed in deionised water, air-dried and stored until further use.

Dewaxed cuticles, or cuticular matrices, were obtained by refluxing the corresponding dry isolated cuticles in chloroform-methanol (1:1) for 12 h. This procedure removes all soluble cuticular lipids. The residual material was kept under dry conditions until further use.

2.2. Cuticular wax isolation

Cuticular waxes were isolated from the following samples: mature grape berries and tomato fruits and leaves of *Agave americana* L., *Araucaria bidwillii* Hook., *C. aurantium* L., *H. helix* L. and *Musa paradisiaca* L. The tissue was washed in water, dried on filter paper, and the cuticular wax was extracted by soaking the fruits or leaves in chloroform for 30 s. The extracts were filtered after mixing the organic solvent with solid anhydrous sodium sulphate. Finally, the organic solvent was evaporated at room temperature and the reconstituted cuticular waxes were stored at room temperature.

2.3. Determination of specific heat

Determination of specific heat of the corresponding cuticular material was performed in a Shimadzu DSC-50 differential scanning calorimeter (DSC, Shimadzu, Japan) with computer-aided data analysis. All experiments followed the same protocol. To establish a baseline, the programme was carried out on an empty pan. The temperature range studied was from 273 K to 338 K at a scanning rate of 5 K min^{-1}. This procedure is then repeated, with a

weighed sample added to the sample holder. The heat flow into the sample is calculated using the following formula:

$$dH/dt = mC_p dT/dt \quad (1)$$

where dH/dt is the heat flow rate (J min^{-1}), m is the sample mass (g), C_p is the specific heat (J K^{-1} g^{-1}) and dT/dt is the scan rate (K min^{-1}).

In order to use Eq. 1 for a specific heat calculation, the ordinate calibration and the temperature programme rate must be known. However, these two parameters may be eliminated from the calculation if a material with a known specific heat is used to calibrate the instrument. One material which can be used to calibrate the instrument is aluminium oxide, Al_2O_3, of which the specific heat is known. Fig. 1 illustrates this method. Thus, the calculation requires only the comparison of two ordinate deflections at the same temperature. The sample (y) and Al_2O_3 variations (y^*) with respect to the baseline are calculated using the formula:

$$y = mC_p \quad (2)$$

$$y^* = m^* C_p^* \quad (3)$$

Dividing Eq. 2 by Eq. 3 and rearranging the terms:

$$C_p/C_p^* = m^* y/my^* \quad (4)$$

For each sample, C_p was calculated in the above mentioned temperature range. In most cases, C_p variation with temperature follows a quadratic equation:

$$C_p = a + bT + cT^2 \quad (5)$$

3. Results and discussion

3.1. Specific heat of plant waxes

Fig. 2 shows the specific heat variation, C_p, of isolated cuticular waxes from different plants in a temperature range from 0°C (273 K) to 45°C (318 K). Thus, this figure shows the specific heat variation of cuticular waxes isolated from the leaves of *H. helix* and *A. bidwillii* and from *V. vinifera* berries (Fig. 2A) and from *C. aurantium* and *M. paradisiaca* leaves and *L. esculentum* fruits (Fig. 2B). In all cases,

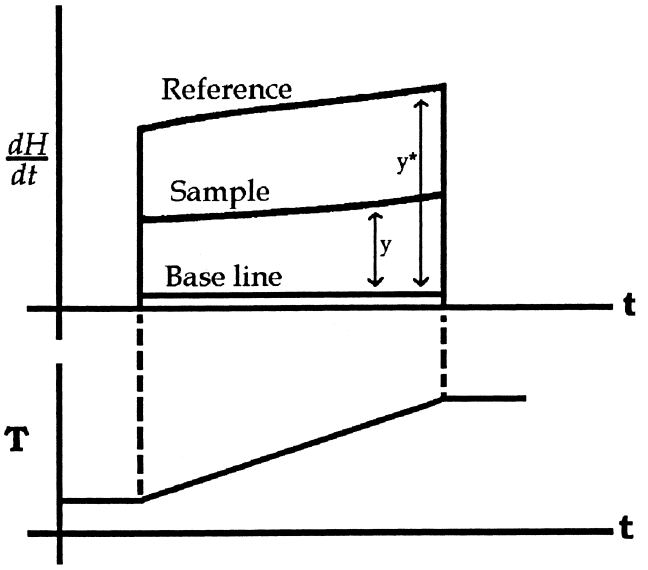

Fig. 1. Linear temperature programme (bottom) and baseline, calibration and sample analysis in the C_p calculation by the ratio method (top). For details, see text.

C_p values showed a small increase with temperature following a good fitting (better than 0.99 in all cases) to the curve represented by Eq. 5. For all cuticular waxes the C_p value range oscillated from 1.5 J K^{-1} g^{-1} (at 273 K) to 3 J K^{-1} g^{-1} (at 318 K).

Moreover, the C_p of individual cuticular wax components was also calculated. Fig. 3 shows the C_p variation of the long chain fatty acid tetracosanoic acid and the *n*-alkane eicosane (Fig. 3A), and of the long chain alcohol *n*-hexacosanol and the long chain aliphatic wax ester of 42 carbon atoms (Fig. 3B). These wax components were elected in this study because they represent the main chemical families of plant cuticular waxes and because these lipid compounds are commonly found in the composition of a wide spectrum of plant waxes [4]. For these compounds, C_p values oscillated from 1.7 J K^{-1} g^{-1} (at 273 K) to 2.5 J K^{-1} g^{-1} (at 318 K). After comparison of Figs. 2 and 3, we can infer that C_p values obtained for the individual components are very similar to those obtained for the isolated and reconstituted cuticular waxes of different plants.

3.2. Specific heat of isolated plant cuticles and cuticular matrices

The specific heat of isolated cuticles from *V. vin-*

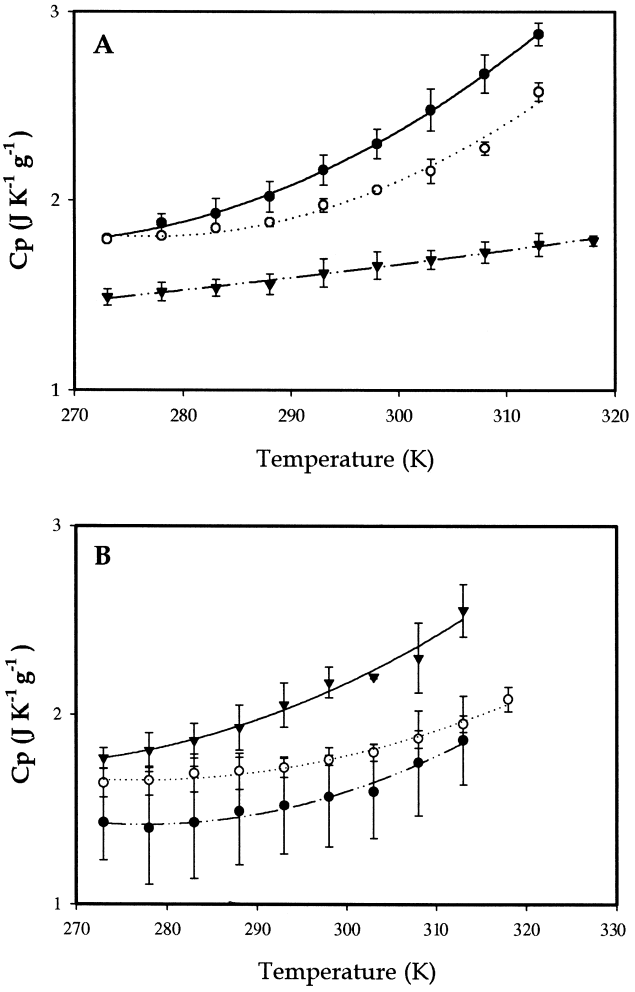

Fig. 2. Specific heat of cuticular waxes isolated from (A) *H. helix* leaves (●), *A. bidwillii* leaves (○) and *V. vinifera* berries (▼); (B) *C. aurantium* leaves (●), *M. paradisiaca* leaves (○) and *L. esculentum* fruits (▼). Bars indicate the standard deviation of three different samples.

ifera berries and *L. esculentum* fruits and from *C. aurantium* leaves was also determined. Fig. 4 shows the C_p variation with temperature of the isolated cuticles of *C. aurantium* leaves (Fig. 4A) and *V. vinifera* berries (Fig. 4B). In addition, for each type of cuticle, the C_p variations of the cuticular matrix (isolated cuticle after wax extraction) and of their corresponding cuticular waxes were also determined and represented in the figures. *L. esculentum* cuticles (data not shown) showed a similar behaviour to the above. These cuticular membranes have been selected because they are well characterised and studied cuticles [1].

The obtained data revealed that the cuticle and its corresponding cuticular matrix have similar specific heat values. This is a clear indication of the low amount of waxes usually present in the cuticles of leaves and fruits [4]. In addition, it is important to emphasise that individual C_p values of the different isolated cuticles and the corresponding cuticular matrices were higher than for cuticular waxes.

From the above results two observations can be made. Significantly high C_p values of cuticular waxes, cuticular wax components, isolated cuticles and cuticular matrices were observed. C_p values of homogeneous and heterogeneous material are low (Figs. 2–4). Cellulose, the main component of plant

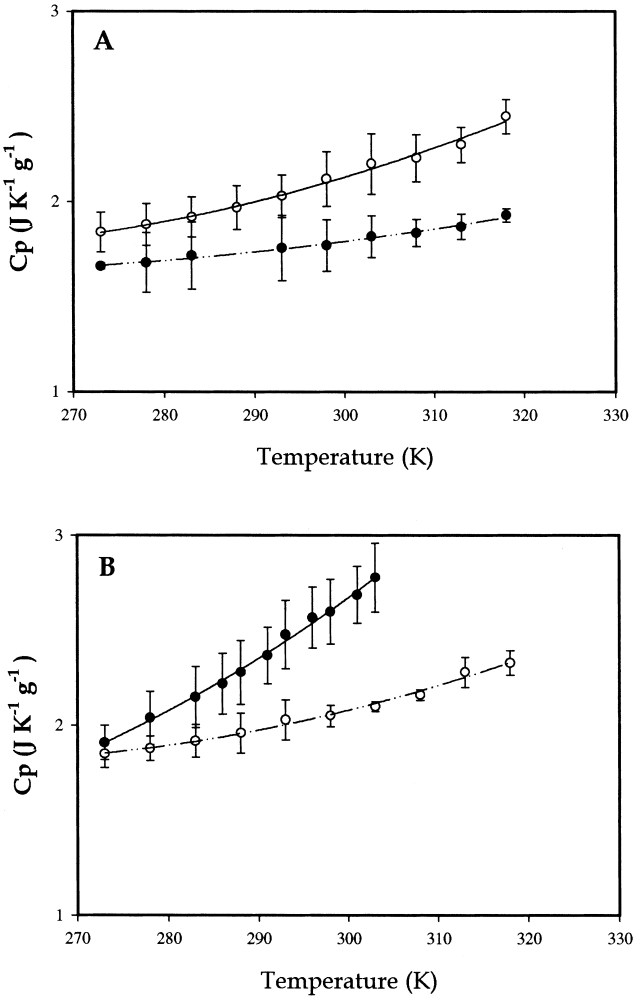

Fig. 3. Specific heat of individual wax components. (A) Tetracosanoic acid (●) and the eicosane (○); (B) *n*-hexacosanol (●) and the long chain wax ester of 42 carbon atoms (○). Bars indicate the standard deviation of three different samples.

cell walls, has a C_p value of 1.55 J K^{-1} g^{-1}, at 293 K, whereas dextrine has a C_p value of 1.30 at 293 K [14]. On the other hand, commercial paraffins have a C_p value of 2.8 at 293 K [14] which is similar to the C_p values of the cuticular components. The high values of this thermodynamic parameter are a consequence of the high cohesion at the molecular level of cuticular components [15]. These facts define the plant cuticle, the interface between the epidermal plant cells and the environment, as a macroscopic lipid barrier with important thermal properties. In their natural environments, plants are sometimes exposed to temperatures of 45–50°C [16]. In spite of the fact that in most plant species cuticular material contributes only minor mass fractions to whole leaves and fruits, the plant cuticular membrane could function as a thermoregulator in the course of the biophysical interaction between plants and the environment. The role of plant cuticle as outer barrier reinforces this physical property.

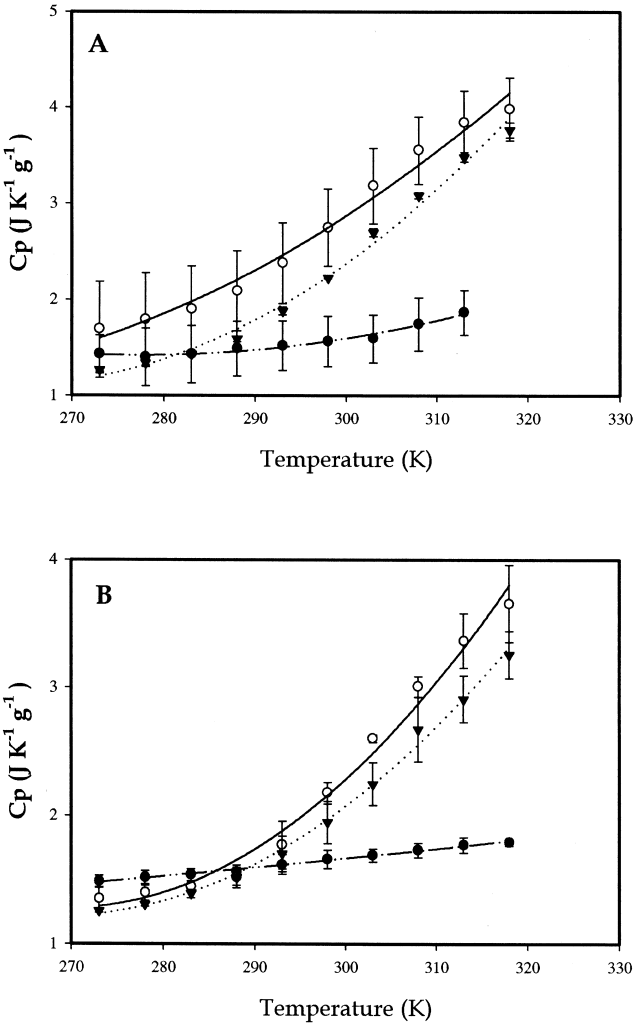

Fig. 4. Specific heat of plant isolated cuticles and their components. (A) Isolated cuticle (●), cuticular waxes (○) and cuticular matrix (▼) of *C. aurantium* leaves. (B) The same as A of *V. vinifera* berries. Bars indicate the standard deviation of three different samples.

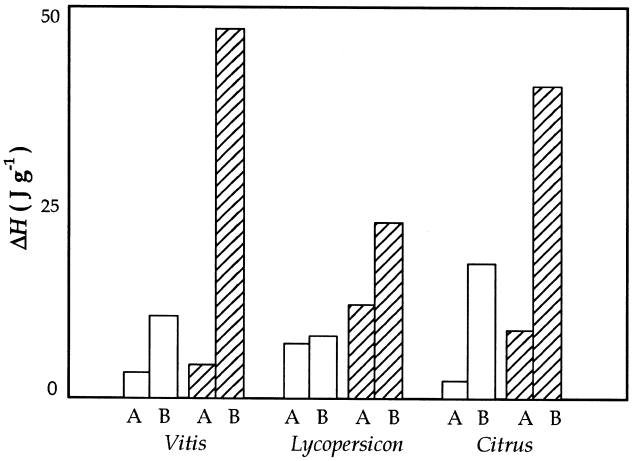

Fig. 5. Variation of ΔH (J g^{-1}) of samples of cuticular waxes (open bars) and isolated cuticles (cross-hatched bars) from grape berries (*V. vinifera* L.), tomato fruits (*L. esculentum* L.) and *C. aurantium* leaves. Temperature intervals are: (A) 273–293 K and (B) 293–318 K.

3.3. C_p values reveal second order phase transitions in plant cuticles

C_p value variations at different temperatures of the isolated cuticles showed an interesting characteristic. There was a gradual increase in the C_p value in the temperature range of 0°C (273 K)–20°C (293 K), but a sharp increase from 20°C (293 K) up to 45°C (318 K).

From a molecular point of view, the rise in C_p just at the last temperature interval (293–318 K) implies an amount of the number of hydrocarbon chain vibrations that mainly form the structure of cutin and cuticular waxes [13]. Consequently, conformational changes in the macromolecular chains occur. These conformational changes are represented, from the corresponding C_p data, by the enthalpy variation, ΔH. Fig. 5 shows the calculated ΔH (from the equation $dH = C_p(T)dT$) of the isolated cuticles and cuticular waxes of *V. vinifera*, *L. esculentum* fruits and *C.*

aurantium leaves. In all cases, the enthalpy variation of the two temperature intervals mentioned above was calculated from the corresponding quadratic equation (5). The ΔH values ranged from 22.5 J g^{-1} in the case of the isolated cuticle of tomato fruit to 47.25 J g^{-1} for the isolated cuticle of grape berry. It is important to point out that these ΔH variations were higher in the case of the isolated cuticles in comparison to the cuticular wax samples only in the temperature range of 20°C (293 K)–45°C (318 K).

Schreiber and Schönherr [11] observed that the expansion coefficients of some isolated plant cuticles were significantly higher in the above mentioned temperature range of 20°C (293 K)–45°C (318 K). Recently Luque and Heredia [12] measured weak secondary thermal transitions in isolated cuticles of tomato fruit in the temperature range from 30°C (303 K) to 70°C (343 K); these transitions are assigned to the cutin biopolymer. Taking into account these considerations, the ΔH values reported in this work can be assigned to the second order phase transitions that occur in the polymeric network of plant cuticles.

The presence of second order transitions in the cuticles implies the existence of conformational changes in the amorphous macromolecular arrangement of the components of plant cuticular membranes [12,13]. It explains the important physical characteristics of these lipophilic barriers, especially if we consider that the plants in their natural environments are exposed to a temperature range similar to that used in the present studies. Thus, the thermally induced structural changes in the cuticle could explain the increased water permeability observed for most plant cuticles above 30–35°C [11]. In addition, these structural changes could determine the mechanical and rheological properties of this biological barrier modulating the mass transfer between the environment and the plant cell. Further research on specific heat variation on a more wide spectrum of plant cuticles and the exact contribution of the cutin in the observed secondary phase transitions will be necessary to complete the biophysical characterisation of this unique biopolymer.

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