Available online at www.sciencedirect.com
SCIENCE DIRECT®
Thermochimica Acta 409 (2004) 165–168
thermochimica acta
www.elsevier.com/locate/tca

Phase transitions in the biopolyester cutin isolated from tomato fruit cuticles

Antonio J. Matas [b], Jesús Cuartero [b], Antonio Heredia [a,*]

[a] *Departamento de Biología Molecular y Bioquímica, Facultad de Ciencias, Grupo de Caracterización y Síntesis de Biopolímeros Vegetales, Universidad de Málaga, E-29071 Málaga, Spain*
[b] *Estación Experimental La Mayora, Consejo Superior de Investigaciones Científicas (CSIC), E-29750, Algarrobo-Costa, Spain*

Received 8 January 2003; received in revised form 8 May 2003; accepted 17 June 2003

Abstract

The specific heat of isolated tomato fruit cuticles and their corresponding cutins have been measured by first time for the physiological temperature in the range of 0–55 °C. Variation of specific heat of the different isolates during fruit growth have been also measured. Isolated cuticles and cutin from young tomato fruits presented a clear glass transition temperature around 23 °C. Water sorption on cutin samples shifted the glass transition temperature to 16.3 °C indicating a clear plasticization of the biopolymer. The presence of these second-order transitions in these lipophilic plant material that act as a molecular barrier between the atmosphere and the plant cell, determine the mechanical and rheological properties of this biological barrier modulating the mass transfer between the environment ant the plant cell.

Keywords: Plant cuticle; Plant cutin; Glass transition; Rheological properties

1. Introduction

A continuous extra-cellular layer, the cuticle, covers aerial parts of higher plants. The plant cuticle is chemically heterogeneous in nature, basically consisting of a wax fraction, soluble in organic solvents, and an insoluble cuticular matrix, the cutin, that forms the framework of the cuticle. The biopolyester cutin is mainly formed by inter-esterification of C_{16} and C_{18} hydroxyalkanoic acids [1]. Structural and physico-chemical studies on plant cutin, including spectroscopical and X-ray diffraction analyses, have been previously reported [2,3]. All data suggest that cutin is an amorphous cross-linked polyester.

A few years ago our research group reported various temperature-dependent changes in isolated plant cuticles and the interactions between their different components, which are predominantly waxes and cutin [4]. Briefly, we reported the occurrence of a glass transition temperature in isolated tomato fruit cutin at subzero temperatures together a weak secondary phase transition in isolated cuticles around 30 °C. These results agreed with previously published data by Schreiber and Schönherr [5] who, calculating volume expansion coefficients, found that the isolated cuticles of several species exhibited second-order phase transitions between 40 and 50 °C. More recently, we discussed the biological implications of these secondary phase transitions after determining the heat capacity of isolated cuticles and dewaxed isolated cuticles from different plants [6]. The thermal characteristics control mechanical behaviour and other properties of these complex biopolymers that act as a barrier between the plant epidermal cell and the atmosphere. In the present paper we report by first time the exact contribution of isolated and purified cutin, the main component of the plant cuticle, in the thermal behaviour of tomato fruit cuticles as well its variation during fruit growth. The effect of water sorption on these properties has been also described.

2. Experimental

2.1. Plant material

Fruit cuticular membranes were enzymatically isolated from greenhouse-grown tomato fruits, cv Cascade

* Corresponding author. Tel.: +34-952-131-940; fax: +34-952-132-000.
E-mail address: heredia@uma.es (A. Heredia).

0040-6031/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0040-6031(03)00357-5

(*Lycopersicon esculentum* Mill.) using an aqueous solution of a mixture of fungal origin cellulase (0.2% w/v) and pectinase (2% w/v). After 4–7 days the cuticles were separated from the epidermis. Cuticular waxes were removed by refluxing the dry fruit cuticles in chloroform–methanol (1:1) for 12 h. Cutin was obtained after exhaustive hydrolysis of the cuticle isolates in a solution of HCl 6 N at 110 °C during 18 h. The residual material was kept under dry conditions until further use.

2.2. Differential scanning calorimetry

The variation of heat capacity (C_p) in samples of cuticular membranes and purified cutin isolated from tomato fruits has been measured using differential scanning calorimetry with computer-aided data analysis (DSC, Shimadzu Corp., model DSC-50, Kyoto, Japan), with temperatures ranging from 0 to 60 °C, following the procedure previously described by Casado and Heredia [6]. 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 0 to 60 °C at the scanning rate of 3 °C 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 equation:

$$\frac{dH}{dt} = mC_p\frac{dT}{dt}$$

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

In order to use the above equation for a specific heat calculation, the ordinate calibration and the temperature programme rate must be known. These two parameters may be eliminated from the calculations using a material with a know specific heat. In this case aluminium oxide was used for this purpose. On the other hand, temperature calibration of the calorimeter was achieved using a sample of indium.

2.3. Water sorption

Water sorption of cutin samples isolated from cuticles of tomato fruits was achieved in a small closed chamber containing a oversaturated solution of monosodium phosphate (98% relative humidity) at room temperature. The amount of water sorbed after 10 h was practically constant. This amount of water was determined by differences of weight between the corresponding samples. DSC experiments with hydrated samples were made using sealed aluminium pans.

3. Results and discussion

Fig. 1 shows the specific heat variation, C_p, of the isolated and purified cutin from young and ripe tomato fruits in a temperature range from 0 to 55 °C. Previously, the chemical characteristics of the membranes were checked by Fourier transform infrared spectroscopy (FT-IR) as described previously [2]. C_p values obtained for cutin from ripe tomato fruit cuticles showed a small increase with temperature (Fig. 1). On the other hand, C_p values measured in the case of young tomato fruit cutin samples showed a different behaviour: the variation of C_p with temperature showed a gradual increase

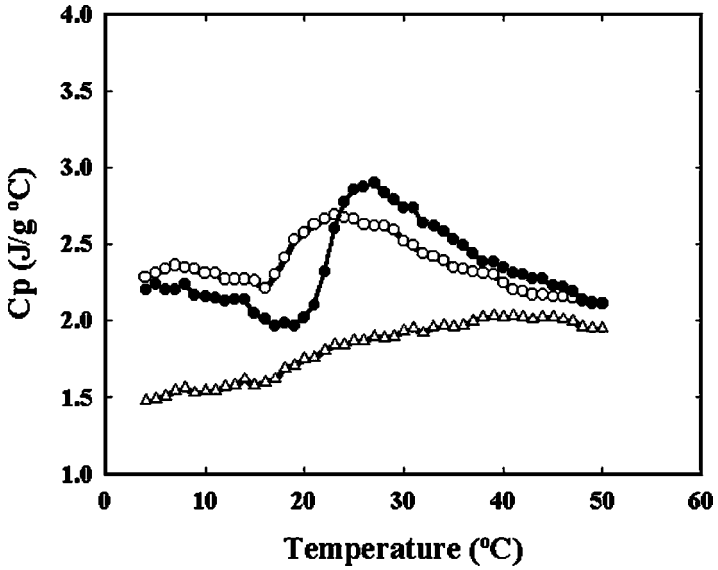

Fig. 1. The temperature dependence of the specific heat capacity (C_p) of a sample of dry purified young tomato fruit cutin (14 days after anthesis) (●), the same sample containing a 5.5% (w/w) of water sorbed (○) and a sample of mature red tomato fruit cutin (49 days after anthesis) (△). C_p was calculated based upon the data of the corresponding thermograms recorded following the methodology described in Section 2 and previously reported by Casado and Heredia [6].

Table 1
Measured glass transition temperatures (T_g) of isolated cuticles (CM) and cutins (CUT) of tomato fruits (*L. esculentum* Mill., cv Cascade) at different growth stages[a]

Fruit characteristics		T_g (°C)	
Days after anthesis	Transversal diameter (cm)	CM	CUT
14	20.1 (2.2)	25.2 (1.9)	23.2 (1.1)
21	27.7 (1.8)	23.6 (3.2)	18.6 (0.1)
28	33.0 (2.8)	22.7 (1.3)	21.0 (1.7)
35	37.7 (4.7)	–	22.3 (4.1)
41	39.2 (1.3)	–	21.8 (1.1)
49	39.7 (2.3)	–	–

[a] T_g (mid-point temperature) was calculated at the mid-point of the overall transition. Parenthesis indicates the standard deviation of three different samples. Transversal diameters were determined with at least 25 fruits.

until 20 °C followed by a sharp increase just above 20 °C (see again Fig. 1). This is a typical feature of the onset of a glass transition characterized in this case by the temperature, T_g, of 23.7 °C. Table 1 shows that the existence of a glass transition is a property of cutin isolated from tomato fruits through the different periods of growth with the exception of cutin isolated from ripe fruit tomato. Moreover, isolated cuticles from young and mature green tomato fruits also showed a clear glass transition temperature (Table 1).

Generally, the glass transition can be interpreted as the range of temperatures at which segment motion of macromolecules becomes thermally activated. The T_g of biopolymers increases with chain rigidity and the intensity of both inter- and intra-molecular interactions, including hindrance to internal rotation along the macromolecular chain. Thus, the glass transition represents a pseudo second-order thermodynamic transition manifested by instantaneous solid-like changes in their physical properties, e.g. viscosity, expansion coefficient and heat capacity. The transition is a kinetic and relaxational process of systems out of internal thermodynamic equilibrium, i.e., it is a non-equilibrium state that trends to relax toward a stable equilibrium [7]. The relevance and importance of glass transition temperatures in biological systems is not completely established. In the case of plant cutin, the framework of the cuticle, the existence of a glass transition temperature implies the existence of severe conformational changes in the amorphous macromolecular arrangement of the polyester. Additionally, it is known that second-order transition, in particular the glass transition temperature, marks the onset of segmental mobility for a polymer conditioning their mechanical and rheological properties [8]. The presence of a glass transition in samples of cuticle and cutin isolated from fruits in development suggests the coexistence, in a physiological interval of temperature, of two stages with different structural and viscoelastic characteristics: below the glass transition temperature the polyester would remain with restricted rotational and vibrational freedom in addition to an effective lose of translational motion. It contributes to a major preservation of cuticular membranes in continuous growth. The cutin of these cuticles have a low degree of cross-linking and it could constitute the chemical basis of these physical and physiological characteristics, especially during the fruit growth. It is interesting to note that cuticle and cutin from ripe tomato fruits did not present any glass transition (Table 1). It could be a consequence of the chemical annealing process in the polyester due to the accumulation of other non-lipid compounds such as phenolics (mainly flavonoids in tomato fruits cuticles) and polysaccharides [1] into the molecular arrangement of the cutin polymer. In the case of the isolated cuticles, the presence of epicuticular and intracuticular waxes additionally contribute to the quenching of the glass transition at early stages of development (Table 1).

Fig. 1 also indicates an important characteristic of the cutin glass transition: water absorption of 5.5% dry weight in the sample plasticizes the cutin isolated from young fruits. The glass transition temperature, T_g, shifted to 16.3 °C when the polymer absorbed water. If we consider that isolated cuticles and cutins can sorb variable amount of water as a function of the relative humidity [9,10], the above-mentioned structural changes in the biopolymer and their mechanical and rheological properties will be consequently modified. The plasticizing effect can be described in terms of lowering of the fracture strength, elastic modulus and viscosity of the biopolymer–water mixtures with an increase in plasticizer content. Our data agree well with the recently reported by Round et al. [11] obtained by the atomic force microscope and solid-state nuclear magnetic resonance. These authors indicated that water absorbed by the tomato fruit cutin functions as a plasticizer promoting molecular flexibility that softens the polymer network and thus decreases its elastic modulus [11]. Thus, the plasticizing effect of water may be based in weakening hydrogen bonds and other intermolecular interactions due to the shielding of these mainly attractive forces by water molecules.

Finally we would like to stress the physiological importance of these findings. Because of the cuticle's barrier role, its rheology is of great interest. Factors that affect the rheological properties of the plant cuticle, such as plasticizing by water or exogenous applied chemicals dissolved or dispersed in adjuvant solutions, could modify the permeability of the biopolymer by modulating the mass transfer between the environment and the plant cell. To conclude, further research will be necessary to complete our understanding of the plant cuticle and its relationships with the environment but it is important to stress that accurate characterization of a glassy state in plant cutin is a critical factor for further rheological studies.

References

[1] P.J. Holloway, in: D.F. Cutler, C.L. Alvin, C.E. Price (Eds.), The Plant Cuticle, The Chemical Constitution of Plant Cutins, Academic Press, London, 1982, p. 45.

[2] F.J. Ramírez, P. Luque, A. Heredia, M.J. Bukovac, Fourier transform IR study of enzymatically isolated tomato fruit cuticular membranes, Biopolymers 32 (1992) 1425–1429.
[3] P. Luque, S. Bruque, A. Heredia, Water permeability of isolated cuticular membranes: a structural analysis, Arch. Biochem. Biophys. 317 (1995) 417–422.
[4] P. Luque, A. Heredia, The glassy state in isolated cuticular membranes: differential scanning calorimetry of tomato fruit cuticular membranes, Plant Physiol. Biochem. 35 (1997) 251–256.
[5] L. Schreiber, J. Schönherr, Phase transitions and thermal expansion coefficients of plant cuticles. The effects of temperature on structure and function, Planta 182 (1990) 186–193.
[6] C.G. Casado, A. Heredia, Specific heat determination of plant barrier lipophilic components: biological implications, Biochim. Biophys. Acta 1511 (2001) 291–296.
[7] B. Wunderlich (Ed.), Thermal Analysis, Academic Press, London, 1982.
[8] F. Rodríguez (Ed.), Principles of Polymer Systems, Hemisphere Publishing Corporation, New York, 1989.
[9] P.M. Chamel, M. Escoubes, Quantitative determination of water sorption by plant cuticles, Plant Cell Environ. 14 (1991) 87–95.
[10] P. Luque, R. Gavara, A. Heredia, A study of the hydration process of isolated tomato fruit cuticular membranes, New Phytol. 129 (1995) 283–288.
[11] A.N. Round, B. Uan, S. Dang, R. Estephan, R.E. Stark, J.D. Batteas, The influence of water on the nanomechanical behaviour of the plant biopolyester cutin as studied by AFM and solid-state NMR, Biophys. J. 79 (2000) 2767–2791.