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Hydrophobicity of the Peach Fruit Surface

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Whole Plant and Eco-physiology

New insights into the properties of pubescent surfaces: the peach fruit (*Prunus persica* Batsch) **as a model**

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ABSTRACT

The surface of peach cv. 'Calrico' is covered by a dense indumentum, which may serve various protection purposes. With the aim of relating structure to function, the chemical composition, morphology and hydrophobicity of the peach skin was assessed as model for a pubescent plant surface. Distinct physico-chemical features were observed for trichomes versus isolated cuticles. Peach cuticles were composed of 53% cutan, 27% waxes, 23% cutin and 1% hydroxycinnamic acid derivatives (mainly ferulic and *p*-coumaric acids). Trichomes were covered by a thin cuticular layer containing 15% waxes and 19% cutin, and were filled by polysaccharide material (63%) containing hydroxycinnamic acid derivatives and flavonoids. The surface free energy, polarity and work of adhesion of intact and shaved peach surfaces were calculated from contact angle measurements of water, glycerol and diiodomethane. The removal of the trichomes from the surface increased polarity from 3.8 (intact surface) to 23.6%, and decreased the total surface free energy chiefly due to a decrease on its non-polar component. The extraction of waxes and the removal of trichomes led to higher fruit dehydration rates. However, trichomes were found to have a higher water sorption capacity as compared to isolated cuticles. The results show that the peach surface is composed of two different materials which establish a polarity gradient, namely: the trichome network which has a higher surface free energy and a higher dispersive component and the cuticle underneath that has a lower surface free energy and higher surface polarity. The significance of the data concerning water-plant surface interactions is discussed within a physiological context.

INTRODUCTION

Plant surfaces have a key role in the protection against abiotic stress factors, such as water losses, high densities of UV and visible radiation or temperature extremes, but are also crucial as defense barrier against biotic threats such as the attack of pathogens or herbivores (Jeffree, 2006; Stavrianakou et al., 2010; Xia et al., 2010).

The cuticle can be considered a cutinized cell wall, emphasizing the composite and heterogeneous nature of this layer and the physiologically crucial interaction between it and the cell wall underneath (Domínguez et al., 2011). This extra-cellular layer is composed of a polymer matrix with waxes embedded into (intra-cuticular) or deposited onto (epi-cuticular waxes) the surface (Heredia, 2003). On the inner side of the cuticle, cutin is mixed with polysaccharide material from the epidermal cell wall (Domínguez et al., 2011). The cuticle matrix is commonly made of a bio-polyester known as cutin, which is constituted by a network of cross-esterified, hydroxy C_{16} and/or C_{18} fatty-acids (Kolattukudy, 1980; Domínguez et al., 2011). Cuticles from some species may contain an alternative non-saponifiable and non-extractable polymer known as cutan, which yields a highly characteristic series of long chain *n*-alkenes and *n*-alkanes upon flash pyrolysis (Villena et al., 1999; Jeffree, 2006).

Cuticular waxes are generally mixtures of long chain aliphatic molecules (mainly C_{20}-C_{40} *n*-alcohols, *n*-aldehydes, very long-chain fatty-acids and *n*-alkanes) and of aromatic compounds (Jetter and Schäffer, 2001; Suh et al., 2005; Leide et al., 2007; Kosma et al., 2009). Apart from the polymer matrix and the waxes, a variable amount of phenolics may be present in the cuticle either in free form, trapped in the matrix or chemically bound to cutin or waxes by ester or ether bonds (Karabourniotis and Liakopoulos, 2005; Domínguez et al., 2009).

According to Werker (2002), trichomes are defined as unicellular or multi-cellular appendages, which originate from epidermal cells only, and develop outwards on the surface of various plant organs. Trichomes can grow in all plant parts and are chiefly classified as "glandular" or "non-glandular". While non-glandular trichomes are distinguished by their morphology, different kinds of glandular trichomes are established by the secretory materials they excrete, accumulate or absorb (Johnson, 1975; Werker, 2000; Wagner et al., 2004). Non-glandular trichomes exhibit a major

variability in size, morphology, and function. They often occur in plants thriving in dry habitats and are abundant in young organs (Fahn, 1986; Karabourniotis et al., 1995).

The effect of the topography of plant surfaces on the deposition of water and pollutants has been largely studied in association with glabrous, waxy surfaces (Holloway, 1969; Schreiber and Schönherr, 1993; Barthlott and Neinhuis, 1997; Wagner et al., 2003; Brewer and Nuñez, 2007; Koch and Ensikat, 2008). However, assessment of liquid-solid interactions following a strict physico-chemical approach as implemented in membrane science (e.g., Khayet et al., 2003, 2007), has never been attempted within a plant physiological context.

In this study we aimed at characterizing for the first time the physical properties of a model pubescent plant surface, taking into account the structure and function of the indumentum. We selected a highly pubescent plant surface to address the following questions: (1) what is the structure of the peach surface and of the epidermis underneath?, (2) Is the surface a composite material formed by the trichomes and the cuticle, and which is the chemical composition of both surface constituents?, and (3) which effect has the trichome layer on the surface free energy, polarity, work of adhesion and rate of water loss by the fruit?

RESULTS

Topography and structure of the peach epidermis

The intact peach surface is covered by a dense indumentum (0.4 to 1 mm thick), constituted by trichomes of different lengths (from 100 to 1000 μm) (Fig. 1A). When cuticles were enzymatically isolated, most of the longest trichomes fell out (Fig. 1C), reducing the thickness and density of the trichome layer. A few stomata (approximately 3 mm^{-2}) occurred in the epidermis underneath. The enzymatic removal of polysaccharides led to the isolation of a sinuous and continuous cuticle that fully covered the small trichomes (approximately 150 μm long) (Fig. 1D). The mechanical removal of trichomes did not induce any visible damage on the fruit epidermis as observed with the naked eye and by microscopy. The remaining shaved peach surface preserved the small trichomes (see Figs. 6, B, D, F as an example) and had a similar topography to the one observed on enzymatically isolated cuticular membranes.

Examination of hand-cut, intact peach sections (Fig. 2, A to C) by light transmission and fluorescence microscopy indicated that the trichomes were non-glandular and unicellular. Trichomes were deeply rooted into the epidermis and had a thin lumen and thick cell walls. Only a few trichomes darkened during examination, suggesting that the majority of them were dead cells at the stage of ripening when fruits were investigated (data not shown). Observation of intact tissues after the application of 10% KOH as an inducer led to the green-yellow fluorescence of the flavonoids present in the trichomes (blue-light excitation; Fig. 2B) and to the light-blue fluorescence of the simple phenols occurring in the cuticle underneath (UV-light excitation; Fig. 2C).

Thin sections of peach tissues (Fig. 2, D to H) were observed by optical microscopy in combination with different dyes. Tissue treatment with Sudan IV, (Fig. 2D) led to the red staining of the cuticle and of the base of trichomes, which appeared to be strongly cutinized. Peach transversal sections stained with Auramine O and observed with long exposure times revealed that both the epidermis and the trichomes were covered by a lipidic layer giving green-yellow fluorescence when examined under UV-light (Fig. 2E). The peach epidermis was found to be sinuous and uneven, having concave (valleys) and convex (peaks) epidermal areas. A disorganized, multiseariate epidermis of 3 to 4 layers of epidermal cells occurred above one or two layers of hypodermis and the large parenchyma cells (Fig. 2F). Trichomes stained in blue (Fig.2, F to H) and initially developed as elongated epidermal cells.

Chemical composition of trichomes and isolated cuticles

The proportion of the chemical constituents of isolated cuticles and trichomes was assessed by ATR-FTIR. Intact tissues were first analyzed and then subjected to the removal of waxes followed by a process of cutin depolymerization.

The ATR-FTIR spectra of peach cuticles and their corresponding isolates after controlled chemical treatment are shown in Fig. 3. The spectrum of the peach fruit cuticle (Fig. 3A), presented strong features of long-chain aliphatic compounds (i.e., bands assigned to asymmetric and symmetric CH_2 stretching at 2918 and 2849 cm^{-1} and CH_2 bending at 1462 cm^{-1}). Besides, the presence of ester functional groups assigned to cutin was revealed by the 1732 cm^{-1} weak band and by the partially-masked vibrations

197 at 1159 and 1104 cm^{-1} (asymmetrical and symmetrical C-O-C stretching, respectively).
198 The band at 1034 cm^{-1} of medium intensity, was assigned to glycosidic bonds typical of
199 polysaccharides. The band appearing at approximately 1688 cm^{-1} was associated with
200 free carboxylic acid functional groups. Vibrations around 1640 and 1515 cm^{-1} were
201 assigned to the stretching of C=C bonds and the stretching of aromatic rings,
202 respectively. More details about the assignments described above can be found in the
203 literature (e.g., Ramírez et al., 1992; Luque et al., 1995; Villena et al., 2000). Wax
204 extraction from isolated cuticles (Fig. 3B), induced a severe reduction of the aliphatic
205 character and an increase of ester and polysaccharide bands. Finally, ATR-FTIR
206 spectrum of the residue resulting after cutin depolymerization (Fig. 3C), indicated a
207 strong polysaccharide character (bands around 1100 to 1000 cm^{-1}) of the remaining
208 material. Nevertheless, the shift from bands corresponding to ester groups to the
209 spectral region of carboxylate groups indicated the presence of significant amounts of
210 the biopolymer cutan (Villena et al., 1999). The chemical composition of isolated peach
211 fruit cuticles corresponded to: 27% waxes, 20% cutin and 53% of an insoluble residue
212 consisting of a mixture of polysaccharides and cutan.

213 In contrast to the cuticle, the ATR-FTIR spectrum of intact trichomes (Fig.4A),
214 presented typical cell wall characteristics, with a small contribution of aliphatic
215 compounds and esterified material which disappeared after progressive wax and cutin
216 removal (Fig. 4, B and C, respectively). Thus, the trichomes were found to be chiefly
217 made of polysaccharide material (66%), with a lower proportion of waxes (15%) and
218 cutin (19%).

219 Cuticular waxes were extracted from isolated trichomes and cuticles and in both
220 cases the predominant compounds were *n*-alkanes. Trichome waxes contained a 92% of
221 *n*-alkanes, the most abundant compounds being unbranched C_{22} to C_{34} alkanes. The
222 waxes extracted from isolated peach cuticles had also a high *n*-alkane fraction (76%),
223 but the most abundant compounds were C_{23} to C_{29} unbranched and methylated alkanes.
224 An array of fatty acids, and only few primary alcohols were determined as minor
225 constituents of the waxes extracted from trichomes and isolated cuticles.

226 Phenolic compounds released after alkaline hydrolysis from different sub-fractions of
227 peach trichomes or isolated cuticles are shown in the HPLC chromatograms (Fig. 5).
228 These hydrolysates revealed a very specific phenolic compound composition, which

was almost identical between the two fractions (Fig. 5, A and C). Three major cinnamic acid derivatives were determined in both fractions, two of them being *p*-coumaric and ferulic acid while the later fraction also contained a number of minor flavonoids (Fig. 5C). The above hydroxycinnamic acid derivatives were also found in the corresponding fractions of the trichomes as part of a more complex profile (Fig. 5, B and D), although they failed to be the dominant compounds.

The isolated cuticles were nine-fold richer in chloroform extractable wax per unit mass compared to the trichomes. In particular, chloroform isolated wax accounted for 20.2% of the cuticle while only 2.19% of the trichome mass was recovered as chloroform extractable wax. All cuticular fractions were much richer in phenolic compounds compared to the corresponding trichome fractions (Table I). Total phenolics accounted for 2.31% of the cuticular wax, a much higher amount as compared to the trichome wax layer (0.62% of the trichome wax, data not shown). Isolated cuticles contained a 236-fold higher concentration of wax-bound *p*-coumaric acid and 89-fold higher concentration of ferulic acid compared to the trichomes. Phenolic compounds bound to the solid residue were also much more abundant in the cuticles as compared to the trichomes, since the former afforded a 34-fold higher amount of *p*-coumaric acid and 6-fold higher amount of ferulic acid when subjected to alkaline hydrolysis as compared to the hairs (Table I).

Contact angle measurements

An example of the contact angles obtained for drops of the 3 liquids in contact with either an intact or shaved peach surface is provided in Fig. 6. The average values of the measured contact angles together with their standard deviation are summarized in Table II. For the two surfaces, the higher contact angle value was obtained for water, followed by that of glycerol and then diiodomethane. The water contact angles of both samples were similar, whereas differences were detected with regard to glycerol and diiodomethane. These results reflect the hydrophobicity of the intact and shaved peach fruit skin, indicating the hydrophobic character of the material covering the surface of both tissues.

The surface free energy of both peach tissues was determined according to the relations (1) to (3), which are based on the contact angle measurements and on the physical properties of the three liquids (Table III). The total surface free energy per unit area of the shaved peach surface is lower than the values determined for the intact skin. This indicates that the morphology and chemistry of the peach surface is changed after the mechanical removal of the trichome layer.

The degree of surface polarity was calculated as the ratio of the non-dispersive surface energy to the total surface energy (γ^{AB} γ^{-1}). The obtained values are 3.8 % and 23.6 % for intact and shaved peach surfaces, respectively. The shaved peach surface has a relatively high non-dispersive (polar) component and lower dispersive (non-polar) component in comparison with the intact peach skin. By removing the trichome layer, the total surface energy decreased because of the decrease in dispersive surface energy and the increase in non-dispersive surface energy (i.e. the increase of polar groups at the surface of the shaved peach skin).

The work of adhesion for the three liquids was calculated using Equation (3), as shown in Table IV. Both peach surfaces exhibit higher adhesion to diiodomethane, followed by that of glycerol and then water. This indicates that the interactions between phases are mainly dispersive in nature.

Rate of fruit dehydration and material swelling

The effect of removing surface waxes and trichomes in relation to the loss of water by the intact fruit is shown in Fig. 7. The highest rates of water loss were determined for de-waxed peaches (20 % loss after two days) followed by shaved ones (13%), while intact fruits only lost 5% of water over the experimental period.

After a period of 24 h storage at 95% RH, a water sorption capacity of (19.2 ± 2.5) and (9.7 ± 0.6) % was recorded for trichomes and isolated cuticles, respectively. The water sorption capacity of the trichomes was found to be twice as high as that of the isolated cuticles. This can be explained by the high proportion of polysaccharides present in the trichomes, which have a higher water sorption capacity as compared to lipids that are the most abundant fraction of compounds determined in the cuticles (Figs. 3 and 4).

DISCUSSION

The surface of the highly-pubescent peach fruit cv. 'Calrico' was investigated as a model, to assess the relationship between surface chemistry and structure with regard to the hydrophobicity of the material. To our knowledge this is the first report in which the surface free energy, polarity and work of adhesion of two different plant materials have been calculated within a physiological plant science context. The significance of the obtained physical parameters has been complemented with structural and chemical determinations of the outer surfaces to help us understand the trichome layer in eco-physiological terms. This innovative approach provides an array of new opportunities to improve our understanding of plant surface related phenomena.

In commercial peach production, there is a growing fashion to clear the trichomes out of surface of peaches via a brushing process that is applied immediately after harvest, which causes no visible damage to the fruit epidermis. Taking into account that the peach epidermis is covered by two distinct materials, namely the trichome layer and the cuticle underneath, it is suggested that the properties of the fruit surface are governed by the combined effect of the abovementioned layers. Thereby, to evaluate the contribution of each material on the physicochemical properties of the surface, analyses were carried out on enzymatically isolated peach cuticles, mechanically isolated trichomes, intact and shaved peach fruits.

Structure and topography of peach epidermis

The trichomes covering the surface were found to be unicellular and non-glandular. Histological studies revealed that the entire peach surface including the trichomes was covered by a cuticle, and that the base of the trichomes was strongly cutinized as described to occur in leaves of xeromorphic plants (Fahn, 1986). Furthermore, a disorganized multiseriate epidermis was observed underneath the cuticle, as reported for the pomaceous fruit of *Mespilus germanica* (Miller, 1984) and for the peach cv. 'O´Henry' (Crisosto et al., 1994).

Chemical composition of the peach surface

Concerning the chemical constituents of the cuticular membranes, 76% of the material was associated with polymer matrix components, containing a strikingly large proportion of cutan. The occurrence of cutan in apple, pepper and berry fruit cuticles has been recently reported by Johnson et al. (2007) and Järvinen et al. (2010). While the significance of this insoluble and more hydrophobic biopolymer remains unclear both in paleobotanical and eco-physiological terms (Deshmukh et al., 2005; Gupta et al., 2006), it has been suggested that it may be a preserved compound in plants growing in xeromorphic environments (Boom et al., 2005).

In contrast, trichomes were largely composed of polysaccharide material and were covered by a thin cuticular layer containing only cutin as matrix. A higher proportion of wax was extracted from the cuticles as compared to the trichomes. The most abundant compounds in both samples corresponded to *n*-alkanes, as observed in other plant species (Jetter et al., 2006). However, longer chain *n*-alkanes were detected in trichome wax extracts as compared to the cuticles. As minor wax constituents an array of fatty acids were detected, with a predominance of palmitic and stearic acid in the trichomes and palmitic, arachidic and linoleic acid in the isolated cuticles. In the case of cuticular isolates, the presence of such compounds may be due to contamination during the process of cuticle isolation, since they are precursors of the structural cuticular biopolymers that are synthesized and accumulated in the epidermal tissue. Minor fatty acid amounts were recovered in the wax extracted from trichomes as compared to the cuticles. The presence of fatty acids in wax extracts has been described in various studies (Jetter et al., 2006), but there is currently no direct evidence that they are part of the wax fraction and it is more likely that they occur due to contamination from the cells underneath.

Three hydroxycinnamic acid derivatives were the dominant compounds extracted from the cuticular waxes. In particular, *p*-coumaric acid and ferulic acid have been characterized as the primary phenylpropanoids being responsible for the characteristic UV-induced blue fluorescence of surface tissues of several plant species (Lichtenthaler and Schweiger, 1998; Karabourniotis et al., 2001; Liakopoulos et al., 2001; Stavroulaki et al., 2007). Similarly to the results reported for other species, these compounds are not part of the pool of tissue soluble phenolic compounds of peach fruits (Tomás-Barberán et al., 2001) but, instead, are often found covalently-bound to plant biopolymers (Riley

and Kolattukudy, 1975; Kroon and Williamson, 1999). Our results indicate that the majority of phenolic compounds determined were bound to the plant biopolymers in contrast to the amounts extracted either in chloroform or methanol (data not shown).

The same three hydroxycinnamic acid derivatives were also part of a more complex profile determined in trichome hydrolysates. The numerous compounds released in the trichome fractions can be ascribed to the fact that trichomes are more complex than isolated cuticles alone. It is most probable that part of the HPLC profile of both fractions of the trichomes may also originate from extractable compounds deposited in the cell walls (Skaltsa et al., 1994; Karabourniotis et al., 1998; Liakopoulos et al., 2006).

Apart from having an effect on pathogen quiescence (Lee and Bostock, 2007), the waxes and phenols present in non-glandular trichomes and cuticles will act as optical filters of excess solar radiation (Reicosky and Hanover, 1978; Karabourniotis and Bornman, 1999; Pfündel et al., 2006).

Hydrophobicity of the surface within an eco-physiological context

The interactions of plant surfaces with water and solutes have been a matter of scientific interest since long ago (Stone, 1963; Fernández and Eichert, 2009). The effect of surface wetness on plant physiology due to natural phenomena such as dew, fog or mist has been addressed in some investigations (Brewer et al., 1991; Brewer and Schmidt, 1997; Pandey and Nagar, 2003; Hanba et al., 2004; Dietz et al., 2007), and is a topic of growing interest for plant eco-physiology (Limm et al., 2009; Aryal and Neuner, 2010; Limm and Dawson, 2010; Johnstone and Dawson, 2010).

By measuring the contact angle and retention of water drops, Brewer et al. (1991) found three different patterns of wettability of pubescent surfaces on 38 species investigated. With the determination of the contact angle of the three liquids and the calculation of the surface free energy, polarity and work of adhesion of the intact and shaved peach surface, we could go a step further in our understanding of the liquid-solid properties of the indumentum. The surface free energy is a parameter specific for each material and different values were obtained for intact and shaved peach surfaces. The higher Lifshitz-van der Waals surface energy component of the natural surface indicated

the more dispersive (non-polar) character of the trichome surface as compared to the cuticle. This result is supported by the data we obtained that confirmed the presence of longer-chain *n*-alkanes in the waxes extracted from the trichomes as compared to those obtained from isolated cuticles. As a consequence, the surface polarity of the intact peach skin was much lower than after the removal of the trichomes (3.8 versus 23.6%), which indicates that the intact surface has a predominant dispersive component and a lower non-dispersive component. By removing the trichomes from the surface as it is commonly done with commercial peaches prior to their storage and distribution to the market, the total surface free energy is decreased due to the decrease in the Lifshitz-van der Waals component and the increase in the acid-base component. This would imply that the trichomes confer a more non-polar character to the surface and that their removal yields the surface more polar and therefore, more susceptible to the occurrence of interactions with water, and water-soluble compounds and contaminants.

The peach skins analyzed are not super-hydrophobic (θ is not above 150^o; Nosonovsky and Bhushan, 2009), but had high contact angles with water due to the presence of air, to the micro- and nano-rugosity of the surface and to its chemical composition. The trichome layer will increase the roughness and surface area of the fruit. However, after the mechanical removal of the long trichomes a rough surface persisted (Figure 6), and the occurrence of air pockets can also be expected. The occurrence of air chambers and their effect on surface water repellency have been modeled for various synthetic and biological surfaces (Nosonovsky and Bhushan, 2009; Xue et al., 2010).

Our results suggest that the peach surface counts on a double hydrophobic protection: on the one hand, the trichome layer covered by longer chain *n*-alkanes and a lower wax proportion and on the other hand, the cuticle which presents a high amount of more polar waxes, and the hydrophobic cutan as major matrix polymer.

When trying to clarify the major role of the trichome indumentum covering the peach surface with regard to the bi-directional exchange of water, we observed that the removal of waxes and trichomes led to significant water losses over time. Several characters reported for xeromorphic plant tissues, namely, the occurrence of a highly pubescent surface, the multiseriate epidermis, the markedly cutinized base of the trichomes and the presence of cutan as major constituent of the cuticle matrix, made us

think that the selected 'Calanda' peach cultivar may be adapted to the prevailing semi-arid conditions in northeast Spain, which are specially hot and dry during the season of fruit growth and development. Such yellow-flesh peach traits may have been developed and selected in China during the many centuries of cultivation of this fruit species in a potentially similar climatic zone (Li, 1970; Lirong, 2005).

The dense indumentum covering the surface up to 1 mm above can affect the boundary layer surrounding the fruit, but may not be the only factor responsible for the increased transpiration rate of shaved peaches. Some studies performed with leaves of *Olea europaea*, *Tillandsia* species and *Mallotus macrostachyus* failed to find a clear relationship between trichome layers and transpiration (Grammatikopoulos et al., 1994; Benz and Martin, 2006; Kenzo et al., 2008).

Despite the hydrophobic character of the surface of trichomes, we showed that they had a high water sorption capacity due to the presence of polysaccharides, which might lead to the absorption of water under certain environmental conditions as shown by e.g., Grammatikopoulos and Manetas (1994). However, the mechanisms of water absorption by plant surfaces including trichomes, are currently not fully understood (Fernández and Eichert, 2009), and should be further elucidated in the future.

In summary, the surface of the peach fruit cv. 'Calrico' is covered by a dense layer of trichomes and a cuticle underneath that protects it against an array of potential biotic and abiotic stress factors. The two materials offer a dual protection against the entry and chiefly the loss of water by the fruit. On the other hand, the occurrence of a dense indumentum and the presence a considerable amount of phenols and waxes in the surface will contribute to limit the attack of pathogens and to attenuate excess radiation. The hydrophobic properties of the peach surface may also influence the bi-directional diffusion of gases and will determine the contact phenomena of the surface with water, contaminants and pathogens.

MATERIALS AND METHODS

Plant material

All materials analyzed corresponded to ripe, undamaged peaches cv. 'Calrico' harvested by mid September (2009, 2010) from an experimental orchard located in the Bajo Aragón area. The selected 'Calanda' cv. is classified as a late-maturing, non-melting, yellow skin and flesh, cling-stone peach variety.

Cuticles were isolated in a citrate buffer solution (pH 3.5) containing 4% cellulase and 4% pectinase (Novozymes, Bagsvared, Denmark) plus 1mM NaN_3 (8 d extraction period; solution changed twice). Trichomes were isolated by gently scraping the peach surface with a sharp knife.

The dehydration rate of intact versus mechanically-shaved and de-waxed (1 min in 2:1 chloroform methanol, v/v) peaches was determined gravimetrically by storing the fruits at 24 ºC and 50% relative humidity (RH) for 2 d.

Microscopy

Thin, hand-cut cross sections of intact peach surfaces were observed with a Zeiss Axiolab fluorescent microscope. Transversal sections were examined first by light transmission and then under blue (emission of green fluorescence by flavonoids) and UV (emission blue fluorescence by simple phenols) excitation after immersion in a 10% (w/v) solution of KOH for 2 min followed by a thorough distilled water rinse. Filter combinations (exciter filter/chromatic beam splitter/barrier filter) were G365/FT395/LP420 (UV 365 nm excitation) and BP450-490/FT510/LP520 (blue light excitation), (Carl Zeiss Jena GmbH, Germany). Microphotographs were taken using a Cybershot DSCS75 digital camera (SONY Corporation, Japan).

Approximately 2 mm thick peach surface pieces were fixed in a 90% ethanol/water, 5% formol and 5% acetic acid solution, dehydrated and embedded in Historesin (Leica, Heidelberg, Germany). Transversal sections were cut with a microtome and were stained with Toluidine blue, Auramine O and Sudan IV prior to microscopic examination (Nikon E 800, Japan).

Fresh intact and shaved peach surfaces and isolated cuticles were directly examined under a VP-SEM microscope (Hitachi S-3400 N, Tokyo, Japan. Acceleration potential: 15 kV, working distance: 10 to 11 mm).

The density of stomata and length of trichomes was assessed by image analysis of SEM micrographs (Image-Pro Plus 6 Bethesda, USA).

Quantitative and qualitative estimation of chemical components by ATR-FTIR

Waxes from isolated cuticles and trichomes were extracted by refluxing in chloroform:methanol (2:1, v/v) for 4 h. The remaining residue was depolymerized by saponification in 2% NaOH for 24 h under reflux conditions. The residual material was weighed. Percentages were calculated according to the weight loss after extraction.

Infrared spectra of isolated cuticles and trichomes, wax extracts and of the residues remaining after alkaline hydrolysis were obtained with an ATR accessory (MIRacle ATR, PIKE Technologies, USA) coupled to a FTIR spectrometer (FT/IR-4100, JASCO, Spain). All spectra were recorded in the 4000 to 700 cm^{-1} range at 4 cm^{-1} resolution and 25 scans were accumulated.

Extraction and determination of cuticular waxes

Dehydrated cuticles and trichomes (250 mg tissue with 2 replications) were extracted for 5 min in 15 mL chloroform -methanol (2:1, v/v) using an ultrasonic bath. Samples were subsequently homogenized and evaporated to dryness with a rotary evaporator. Then 5 mL of a methanolic NaOH solution (0.5M) were added to the plant solid residue, the mixture being boiled for 10 min using a Vigreux column. When samples were cool, 5mL BF_3-methanol (14% w/w, diluted with water-free methanol) were added and the mixture was boiled for 2 min prior to the addition of 4 mL *n*-heptane. When the samples cooled down again, 15 mL of saturated NaCl (2.5 g L^{-1}) dissolved in ultrapure water (milli Q Plus 185, Millipore) were added and the solutions were homogenized for 15 s. The organic phase was collected (*n*-heptane; 99%, HPLC grade, Scharlau, Spain) and filtered. The composition of the samples was determined by GS-MS (GC Hewlett Packard HP-6890 equipped with an autosampler Combipal and quadrupole mass

spectrometer HP 5973). The chromatographic conditions were as follows (86 min per run): the injection volume was 1 μL (splitless mode), Helium was the carrier gas (1 mL min^{-1}) and the injector and detector temperatures were set to 250 ºC. The column (J&W 122-5532 DB-5ms, AgilentTechnologies) was set to 55 ºC isothermal for 4 min, then increased to 155 ºC at a rate of 5 ºC min^{-1} and held isothermal for 2 min, raised to 320 ºC at a rate of 3 ºC min^{-1} and held isothermal for 5 min. The MS conditions were: 70 eV ionization voltage, 230 ºC ion source temperature; 50–650 units of mass scan range and 5 min wait time. The compounds were identified by comparing their mass spectra with NIST and WILEY275 library spectra, confirming the results by the Kovats index. All standards used were from Sigma-Aldrich, Spain.

Extraction and determination of phenolic compounds

Cuticular and trichome waxes from 1g tissue were extracted in chloroform (5 min) and subjected to alkaline hydrolysis (4 M NaOH, 1 h at 60 °C under a N_2 stream) as described by Liakopoulos et al. (2001). After acidification of the solutions with HCl (pH 1), samples were extracted three times in ethyl-acetate and the combined extracts were extracted with water to remove acid and concentrated in a rotary evaporator at 30 °C. The solid tissue residue after wax removal (STR) was subsequently extracted in methanol (1 mL per 10 mg of material, 1 h in an ultrasonic bath) and methanolic extracts were evaporated to dryness. The remaining STR was subjected to alkaline hydrolysis, acidification and concentration to dryness as described above. All dry residues were re-diluted in 4 or 8 mL 50% methanol and injected into a Zorbax Stablebond SB-C18 column (5 μm particle size; 250 × 4.6 mm; Agilent Technologies, Palo Alto, CA, USA) via a 20 μL loop, connected to a Prominence HPLC equipped with a photodiode array detector operating at 200-800 nm (Shimadzu Corporation, Kyoto, Japan). The column was eluted at 30°C using the following linear gradient: initially: A (1% H_3PO_4):B (MeOH) 75:25; gradient to 70:30 in 10 min; gradient to 65:35 in 7 min; gradient to 0:100 in 3 min; flow rate 1 mL min^{-1}. Chromatograms were captured using LC Solution ver. 1.23 SP1. Phenolic compounds were identified by comparison with pure standards (Extrasynthese S.A., Genay, France). The quantitative determination of *p*-coumaric acid and ferulic acid was based on reference curves at 280 nm.

532 **Water sorption of cuticles and trichomes**

533 The water sorption capacity of trichomes and isolated cuticles was measured
534 gravimetrically. Tissues (55 and 65 mg) were dried for 24 h in a desiccator at very low
535 RH (silica gel). The samples were subsequently kept in a closed chamber for 24 h at
536 95% RH, which was achieved by exposure to a supersaturated solution of $Pb(NO_3)_2$ at
537 25 ºC. The water sorption capacity was calculated by measuring the weight increment of
538 dehydrated and water saturated tissues.

539

540 **Contact angle measurement and estimation of surface free-energy**

541 The advancing contact angles of three liquids, i.e., double-distilled water, glycerol
542 (99% purity, Sigma-Aldrich), and diiodomethane (99% purity, Sigma-Aldrich) were
543 measured at ambient temperature (25ºC) using a Drop Shape Analysis System DSA 100
544 (Krüss-GmbH, Hamburg, Germany).

545 Contact angles were determined on intact and shaved peach surfaces (30 repetitions)
546 by placing the baseline tangent to the area of touch between the solid and the liquid as
547 enabled by the measuring device software. In the latter case, trichomes were removed
548 by gently scraping the peach surface with a sharp knife. Skin sections of approximately
549 1 x 0.5 cm^2 and 1 mm thickness were cut with a scalpel. Drops of the different liquids
550 were deposited onto the surface using a manual dosing system with a 3 mL syringe and
551 a 0.5 mm diameter needle. Side view images of the drops were captured at a rate of 10
552 frames s^{-1}. The contact angles were automatically calculated by fitting the captured drop
553 shape to that calculated from the Young–Laplace equation.

554

555 **Theoretical background and calculations based on contact angle determinations**

556 Several data based on contact angle measurements of the three liquids with intact
557 skins or after the removal of the trichomes were obtained by means of some equations
558 (1 to 3). The film surface free energy (or surface tension, γ) components were
559 determined from contact angle measurements using the Lifshitz-van der Waals (LW)
560 method, also known as acid-base (AB) approach or van Oss, Good, and Chaudhury
561 method (van Oss et al., 1987, 1988). The theory behind this method of estimating the

562 solid surface free energy and its components has been extensively described elsewhere
563 (Owens and Wendt, 1969; Mittal, 1993). Van Oss et al. (1987, 1988) divided γ, into
564 different components, i.e. the Lifshitz-van der Waals (*LW*), acid (+) and base (-)
565 components.

566 $$\gamma_i = \gamma_i^{LW} + \gamma_i^{AB} = \gamma_i^{LW} + 2\sqrt{\gamma_i^+ \gamma_i^-} \quad (1)$$

567 where *i* denotes either the solid or the liquid phase. The acid-base (AB) component
568 (γ_i^{AB}) takes into account the electron-donor (γ_i^-) and the electron-acceptor (γ_i^+)
569 interactions. The following expression was given for solid-liquid systems (van Oss et
570 al., 1987, 1988)

571 $$(1+\cos\theta)\gamma_l = 2(\gamma_s^{LW}\gamma_l^{LW})^{1/2} + 2(\gamma_s^+\gamma_l^-)^{1/2} + 2(\gamma_s^-\gamma_l^+)^{1/2} \quad (2)$$

572 where the three components of the surface free energy of the solid, γ_s^{LW}, γ_s^+ and γ_s^- can
573 be determined from the contact angle measurements of three testing liquids with known
574 surface tension components (i.e. water: γ_s^{LW} = 21.8 mJ m^{-2}, $\gamma_s^+ = \gamma_s^-$ = 25.5 mJ m^{-2};
575 glycerol: γ_s^{LW} = 34.0 mJ m^{-2}, γ_s^+ = 3.92 mJ m^{-2}, γ_s^- = 57.4 mJ m^{-2} and diiodomethane:
576 γ_s^{LW} = 50.8 mJ m^{-2}, $\gamma_s^+ = \gamma_s^-$ = 0 mJ m^{-2}).

577

578 In addition, the degree of surface polarity of intact and shaved peach surfaces was
579 calculated as the ratio of the non-dispersive surface energy to the total surface energy
580 ($\gamma^{AB}\gamma^{-1}$).

581 Finally, to discuss liquid-solid interactions the total work of adhesion (W_a; Kwok and
582 Neumann, 1999) was determined for each liquid and type of peach surface, following
583 the equation:

584 $$W_a = \gamma_s + \gamma_{lv} - \gamma_{sl} = (1+\cos\theta)\gamma_l \quad (3)$$

585 where γ_s is the surface free energy of the solid, γ_{lv} is the interfacial tension of the liquid
586 and γ_{sl} corresponds to the interfacial tension between the solid and the liquid.

587

588

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LITERATURE CITED

Aryal B, Neuner G (2010) Leaf wettability decreases along an extreme altitudinal gradient. Oecol **162**: 1-9

Barthlott W, Neinhuis C (1997) Purity of the sacred lotus, or escape from contamination in biological surfaces. Planta **202**: 1-8

Benz BW, Martin CE (2006) Foliar trichomes, boundary layers, and gas exchange in 12 species of epiphytic *Tillandsia* (Bromeliaceae). J Plant Physiol **163**: 648-656

Boom A, Sinninghe Damsté JS, de Leeuw JW (2005) Cutan, a common aliphatic biopolymer in cuticles of drought-adapted plants. Organ Geochem **36**: 595–601

Brewer CA, Smith WK, Vogelmann TC (1991) Functional interaction between leaf trichomes, leaf wettability and the optical properties of water droplets. Plant Cell Environ **14**: 955-962

Brewer CA, Smith WK (1997) Patterns of leaf surface wetness for montane and subalpine plants. Plant Cell Environ **20**: 1-11

Brewer CA, Nuñez CI (2007) Patterns of leaf wettability along an extreme moisture gradient in western Patagonia, Argentina. Int J Plant Sci **168:** 555-562

Crisosto CH, Johnson RS, Luza JG, Crisosto GM (1994) Irrigation regimes affect fruit soluble solids concentration and rate of water loss of 'O'Henry' peaches. HortSci **29**: 1169-1171

Deshmukh AP, Simpson AJ, Hadad CM, Hatcher PG (2005) Insights into the structure of cutin and cutan from *Agave americana* leaf cuticle using HRMAS NMR spectroscopy. Organ Geochem **36**: 1072-1085

Dietz J, Leuschner C, Hölscher D, Kreilein H (2007) Vertical patterns and duration of surface wetness in an old-growth tropical montane forest, Indonesia. Flora **202**: 111-117

Domínguez E, Luque P, Heredia A (2009) Sorption and interaction of the flavonoid naringenin on tomato fruit cuticles. J Agric Food Chem **57**: 7560-7564

Domínguez E, Heredia-Guerrero JA, Heredia A (2011). The biophysical design of plant cuticles: an overview. New Phytol **189**: 938-949

Fahn A (1986) Structural and functional properties of trichomes of xeromorphic leaves. Ann Bot **57**: 631-637

Fernández V, Eichert T (2009) Uptake of hydrophilic solutes through plant leaves: current state of knowledge and perspectives of foliar fertilization. Crit Rev Plant Sci **28**: 36-68

Grammatikopoulos G, Manetas Y (1994) Direct absorption of water by hairy leaves of *Phlomis fruticosa* and its contribution to drought avoidance. Can J Bot **72**: 1805-1811

Grammatikopoulos G, Karabourniotis G, Kyparissis A, Petropoulou Y, Manetas Y (1994) Leaf hairs of olive (*Olea europaea*) prevent stomatal closure by ultraviolet-B radiation. Austral J Plant Physiol **21**: 293-301

Gupta NS, Collinson ME, Briggs EG, Evershed RP, Pancost RD (2006) Reinvestigation of the occurance of cutan in plants: implications for the leaf fossil record. Paleobiol **32**: 432-449

Hanba YT, Moriya A, Kimura K (2004) Effect of leaf surface wetness and wettability on photosynthesis in bean and pea. Plant Cell Environ **27**: 413-421

Heredia A (2003) Biophysical and biochemical characteristics of cutin, a plant barrier biopolymer. Biochim Biophys Acta **1620**: 1-7

Holloway PJ (1969) The effects of superficial wax on leaf wettability. Ann Appl Biol **63**: 145–153.

Järvinen R, Kaimainen M, Kallio H (2010) Cutin composition of selected northern berries and seeds. Food Chem **122**: 137–144

Jeffree CH (2006) The fine structure of the plant cuticle. In M Riederer, C Müller, eds, Annual Plant Reviews **23**: Biology of the Plant Cuticle. Blackwell, Oxford, pp 11-125

Jetter R, Schäffer S (2001) Chemical composition of the *Prunus laurocerasus* leaf surface. Dynamic changes of the epicuticular wax film during leaf development. Plant Physiol **126**: 1725-1737

Jetter R, Kunst L, Samuels AL (2006) Composition of plant cuticular waxes. In M Riederer, C Müller, eds, Annual Plant Reviews **23**: Biology of the Plant Cuticle. Blackwell, Oxford, pp 145-181

Johnson HB (1975) Plant pubescence: an ecological perspective. Bot Rev **41**: 233-258

Johnson EJ, Chefetz B, Xing B (2007) Spectroscopic Characterization of Aliphatic moieties in four plant cuticles. Comm Soil Sci Plant Anal **38**: 2461-2478

Johnstone JA, Dawson TE (2010) Climatic context and ecological implications of summer fog decline in the coast redwood region. Proc Natl Acad Sci USA **107**: 4533-4538

Karabourniotis G, Kotsabassidis D, Manetas Y (1995) Trichome density and its protective potential against ultraviolet-B radiation damage during leaf development. Can J Bot **73:** 376-383

Karabourniotis G, Kofidis G, Fasseas C, Liakoura V, Drossopoulos I (1998) Polyphenol deposition in leaf hairs of *Olea europaea* (Oleaceae) and *Quercus ilex* (Fagaceae). Am J Bot **85**: 1007-1012

Karabourniotis G, Bormann JF (1999) Penetration of UV-A UV-B and blue light through the leaf trichome of two xeromorphic plants, olive and oak, measured by optical fibre microprobes. Physiol Plant **105**: 655-661

Karabourniotis G, Tzobanoglou D, Nikolopoulos D, Liakopoulos G (2001) Epicuticular phenolics over guard cells: Exploitation for in situ stomatal counting by fluorescence microscopy and combined image analysis. Ann Bot **87**: 631-639

Karabourniotis G, Liakopoulos G (2005) Phenolic compounds in plant cuticles: physiological and ecophysiological aspects In A Hemantaranjan, ed, Advances in Plant Physiology vol. 8. Scientific Publishers, Varanasi, pp. 33-47

Kenzo T, Yoneda R, Azani MA, Majid NM (2008) Changes in leaf water use after removal of leaf lower surface hairs on *Mallotus macrostachyus* (Euphorbiaceae) in a tropical secondary forest in Malaysia. J Forest Res **13**: 137-142

Khayet M, Feng CY, Matsuura T (2003) Morphological study of fluorinated asymmetric polyetherimide ultrafiltration membranes by surface modifying macromolecules. J Membr Sci **213:** 159-180

Khayet M, Vazquez Alvarez M, Khulbe KC, Matsuura T (2007) Preferential surface segregation of homopolymer and copolymer blend films. Surf Sci **601**: 885-895

Koch K, Ensikat HJ (2008) The hydrophobic coatings of plant surfaces: Epicuticular wax crystals and their morphologies, crystallinity and molecular self-assembly. Micron **39**: 759-772

Kolattukudy PE (1980) Biopolyester Membranes of Plants: Cutin and Suberin. Science **208** (4447): 990-1000

706 **Kosma DK, Bourdenx B, Bernard A, Parsons EP, Lu S, Joubes J, Jenks MA** (2009)
707 The impact of water deficiency on leaf cuticle lipids of Arabidopsis. Plant Physiol
708 **151**: 1918-1929
709 **Kroon PA, Williamson G** (1999) Hydroxycinnamates in plants and food: current and
710 future perspectives. J Sci Food Agric **79**: 355-361
711 **Kwok DY, Neumann AW** (1999) Contact angle measurement and contact angle
712 interpretation Adv Colloid Interface Sci **81:** 167-249
713 **Lee MH, Bostock RM** (2007) Fruit exocarp phenols in relation to quiescence and
714 development of *Monilinia fructicola* Infections in *Prunus spp*.: A Role for Cellular
715 Redox? Phytopathol **97**: 269-277
716 **Leide J, Hildebrandt U, Reussing K, Riederer M, Vogg G** (2007) The developmental
717 pattern of tomato fruit wax accumulation and its impact on cuticular transpiration
718 barrier properties: effects of a deficiency in a beta-ketoacyl-coenzyme A synthase
719 (LeCER6). Plant Physiol **144**:1667-1679
720 **Li HL** (1970) The Origin of Cultivated Plants in Southeast Asia. Econ Bot
721 **24**: 3-19
722 **Liakopoulos G, Stavrianakou S, Karabourniotis G** (2001) Analysis of epicuticular
723 phenolics of *Prunus persica* and *Olea europaea* leaves: Evidence on the chemical
724 origin of the UV-induced blue fluorescence of stomata. Ann Bot **87**: 641-648
725 **Liakopoulos, G, Stavrianakou S, Karabourniotis G** (2006) Trichome layers versus
726 dehaired lamina of Olea europaea leaves: differences in flavonoid distribution, UV-
727 absorbing capacity, and wax yield. Env Exp Bot **55**: 294-304
728 **Lichtenthaler HK, Schweiger J** (1998) Cell wall bound ferulic acid, the major
729 substance of the blue-green fluorescence emission of plants. J Plant Physiol **152**:
730 272-282
731 **Limm EB, Simonin KS, Bothman AG, Dawson TE (**2009) Foliar water uptake: A
732 common water acquisition strategy for plants of the redwood forest. Oecol **161**: 449-
733 459
734 **Limm EB, Dawson TE (**2010) *Polystichum munitum* (Dryopteridaceae) varies
735 geographically in its capacity to absorb fog water by foliar uptake within the
736 redwood forest ecosystem. Am J Bot **97**: 1121-1128
737 **Lirong W** (2005) Status report on genetic resources of peach in East Asia. IPGRI
738 Surveying Rep, 44 p.
739 **Luque P, Bruque S, Heredia A** (1995) Water permeability of isolated cuticular
740 membranes: a structural analysis. *Arch Biochem Biophys* **317**: 417-422
741 **Miller RH** (1984) The multiple epidermis-cuticle complex of medlar fruit *Mespilus*
742 *germanica* L. (Rosaceae). Ann Bot **53**: 779-792
743 **Mittal KL** (1993) Contact Angle, Wettability and Adhesion. VSP, Utrecht, The
744 Netherlands
745 **Nosonovsky M, Bhushan B** (2009) Superhydrophobic surfaces and emerging
746 applications: non-adhesion, energy, green engineering. Curr Opin Coll Interf Sci **14**:
747 270-280
748 **Owens DK, Wendt RC** (1969) Estimation of the Surface Free Energy of Polymers. J
749 Appl Polym Sci **13**:1741-1747
750 **Pandey S, Nagar PK** (2003) Pattern of leaf surface wetness in some important
751 medicinal and aromatic plants of western Himalaya. Flora **198**: 349-357
752 **Pfündel EE, Agati G, Cerovic ZG** (2006) Optical properties of plant surfaces. In M
753 Riederer, C Müller, eds, Annual Plant Reviews **23**: Biology of the Plant Cuticle.
754 Blackwell, Oxford, pp 216-249

Ramírez FJ, Luque P, Heredia A, Bukovac MJ (1992) Fourier transform IR study of enzymatically isolated tomato fruit cuticular membrane. *Biopolym* **32**: 1425-1429

Reicosky DA, Hanover JW (1978) Physiological effects of surface waxes. Plant Physiol **62:** 101–104.

Riley RG, Kolattukudy PE (1975) Evidence for covalently attached *p*-coumaric acid and ferulic acid in cutins and suberins. Plant Physiol **56**: 650-654

Schreiber L, Schönherr J (1993) Contact areas between leaf surfaces and aqueous solutions: quantitative determination of specific leaf surface contact areas. J Exp Bot **44**: 1653-1661

Skaltsa H, Verykokidou E, Harvala C, Karabourniotis G, Manetas Y (1994) UV-B protective potential and flavonoid content of leaf hairs of *Quercus ilex*. Phytochem **37**: 987-990

Stavrianakou S, Liakopoulos G, Miltiadou D, Markoglou AN, Ziogas BN, Karabourniotis G (2010) Antifungal and Antibacterial Capacity of Extracted Material from non-Glandular and Glandular Leaf Hairs Applied at Physiological Concentrations. Plant stress **4**: 25-30

Stone EC (1963) Ecological importance of dew. Quart Rev Biol **38**: 328-341

Suh MC, Samuels AL, Jetter R, Kunst L, Pollard M, Ohlrogge J, Beisson F (2005) Cuticular lipid composition, surface structure, and gene expression in Arabidopsis stem epidermis. Plant Physiol **139**:1649-1665

Tomás-Barberán FA, Gil MI, Cremin P, Waterhouse AL, Hess-Pierce B, Kader AA (2001) HPLC - DAD - ESIMS analysis of phenolic compounds in nectarines, peaches, and plums. J Agric Food Chem **49**: 4748-4760

van Oss CJ, Chaudhury MK, Good RJ (1987) Monopolar surfaces. Adv Colloid Interf Sci **28**: 35-64

van Oss CJ, Chaudhury MK, Good RJ (1988) Interfacial Lifshitz-van der Waals anpolar interactions in macroscopic systems. Chem Rev **88**: 927-941

Villena JF, Domínguez E, Stewart D, Heredia A (1999) Characterization and biosynthesis of non-degradable polymers in plant cuticles. Planta **208**: 181–187

Villena FJ., Domínguez E., Heredia A. (2000) Monitoring biopolymers present in plant cuticles by FT-IR spectroscopy. J Plant Physiol **156:** 419-422

Wagner P, Fürstner R, Barthlott W, Neinhuis C (2003) Quantitative assessment to the structural basis of water repellency in natural and technical surfaces, J Exp Bot **54**: 1295-1303

Wagner GJ, Wang E, Shepherd RW (2004) New approaches for Studying and Exploiting an old protuberance, the plant trichome. Ann Bot **93**: 3-11

Werker E (2000) Trichome diversity and development. Adv Bot Res **31**: 1-35

Xia Y, Yu K, Navarre D, Seebold K, Kachroo A, Kachroo P (2010) The glabra1 mutation affects cuticle formation and plant responses to microbes. Plant Physiol **154**: 833-846

Xue CH, Jia ST, Ma JZ (2010) Large-area fabrication of superhydrophobic surfaces for practical applications: an overview. Sci Technol Adv Mater 11:1-15

TABLES

Table I. *Concentration of major phenolic compounds released after alkaline hydrolysis from different fractions extracted from trichomes and isolated cuticles*

Material	Fraction	Compound	Concentration (µg g^{-1} material)
Trichomes	Chloroform isolated wax	*p*-coumaric acid	8.97
		ferulic acid	6.37
	Solid residue	*p*-coumaric acid	27.9
		ferulic acid	46.5
Isolated cuticle	Chloroform isolated wax	*p*-coumaric acid	2120
		ferulic acid	567
	Solid residue	*p*-coumaric acid	957
		ferulic acid	278

Table II. *Contact angles of water (θ_w), glycerol (θ_g) and diiodomethane (θ_d) on intact and shaved peach fruit surfaces*

Sample	θ_w (º)	θ_g (º)	θ_d (º)
Intact	134.2±7.0	130.9±7.0	55.7±3.9
Shaved	134.5±7.0	117.9±4.9	80.3±7.5

Table III. *Surface free energy per unit area. Lifshitz van der Waals component(γ^{LW}), Acid-base component (γ^{AB}) with the contribution of electron donor(γ^{-}) and electron acceptor(γ^{+}) interactions, total surface free energy (γ) and surface polarity ($\gamma^{AB}\ \gamma^{-1}$) for intact and shaved peach fruit surfaces*

Sample	γ^{LW} (mJ m^{-2})	γ^{-} (mJ m^{-2})	γ^{+} (mJ m^{-2})	γ^{AB} (mJ m^{-2})	γ (mJ m^{-2})	$\gamma^{AB}\ \gamma^{-1}$ *(%)*
Intact	31.06	0.04	10.03	1.22	32.28	3.8
Shaved	17.37	0.99	7.26	5.37	22.73	23.6

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Table IV. *Work of adhesion of intact and shaved peach surfaces for water (Ww), glycerol (Wg) and diiodomethane (Wd).*

Sample	$W_{a,w}$ (mJ m^{-2})	$W_{a,g}$ (mJ m^{-2})	$W_{a,d}$ (mJ m^{-2})
Intact peach	22.0	22.1	79.4
Shaved peach	21.8	34.0	59.40

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FIGURE LEGENDS

Figure 1. Scanning electron micrographs of peach intact surfaces (A, B) and isolated cuticles (C,D). (A) Intact peach surface (x100). (B) Stoma observed in an intact surface after the mechanical removal of trichomes (x450). (C) Upper surface of an isolated cuticle (x100). (D) Lower surface of an isolated cuticle (x2000).

Figure 2. Micrographs of transversal peach fruit sections of: (A) intact tissue observed by light transmission, (B) intact tissue treated with the inducer and observed under blue light excitation, (C) intact tissue with the inducer and observed under UV-light excitation, (D) embedded tissue stained with Sudan IV, (E) embedded tissue stained with Auramine O and UV-light, and (F, G, H) embedded tissue stained with Tolouidine blue.

Figure 3. ATR-FTIR spectra of: (A) isolated peach fruit cuticles, (B) isolated cuticles without waxes, and (C) residue after chemical depolymerisation of cutin. Spectra (B) and (C) show significant losses of aliphatic components and a higher presence of polysaccharides. The arrow in (C) indicates a shift from ester to carboxylate groups, showing the presence of cutan.

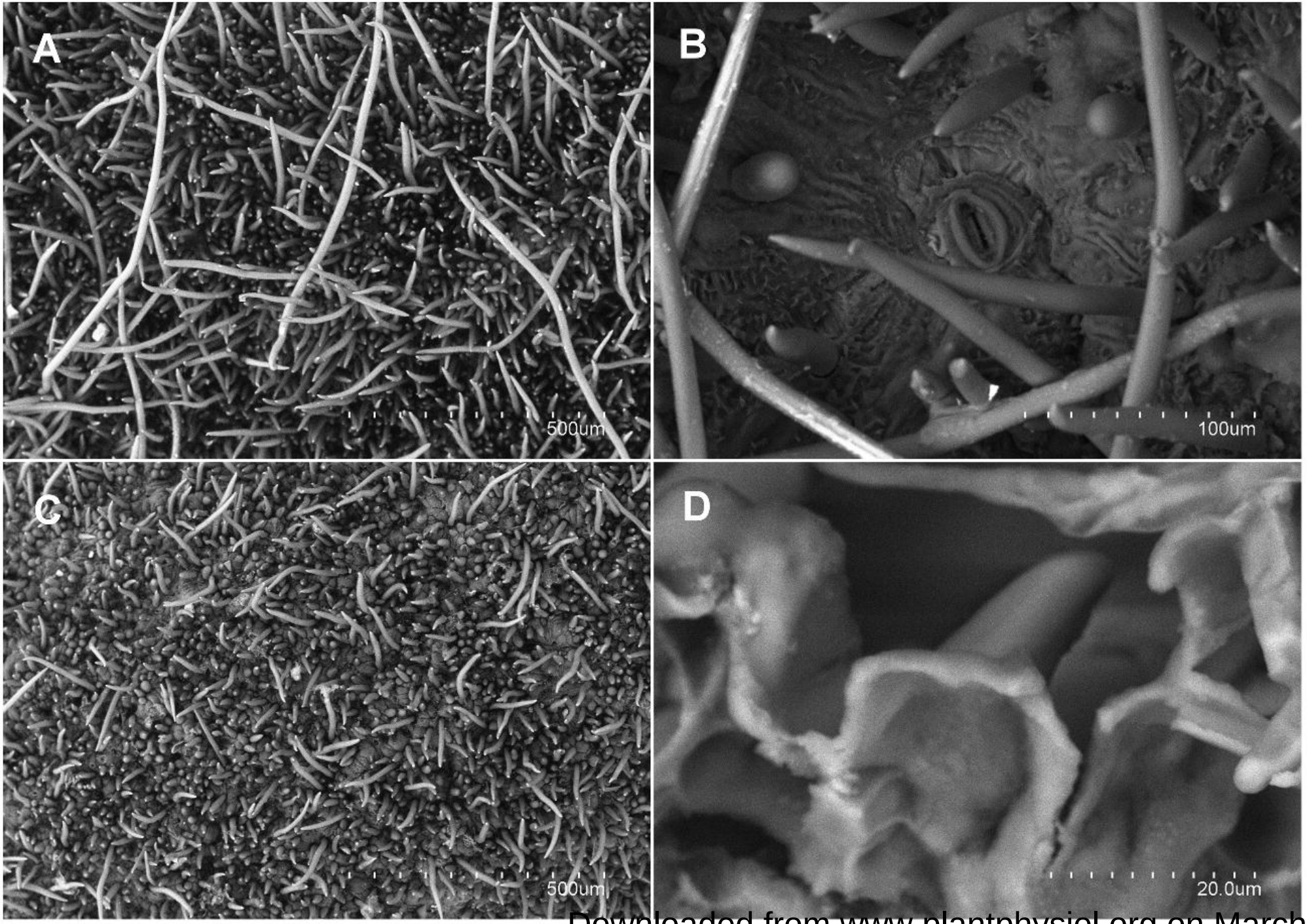
Figure 4. ATR-FTIR spectra of: (A) isolated peach fruit trichomes, (B) isolated trichomes without waxes, and (C) residue after depolymerisation of cutin. The overall spectra have a strong polysaccharide character, typical of cell wall isolates.

Figure 5. Chromatograms of: (A) Hydroxycinnamic acid derivatives (*p*-coumaric acid, ferulic acid and unidentified HC peak) released from alkaline hydrolysis of chloroform isolated cuticular waxes. (B) Hydroxycinnamic acid derivatives and flavonoid (unidentified FL peak) released from alkaline hydrolysis of chloroform isolated trichome waxes. (C) Hydroxycinnamic acid derivatives and flavonoids (unidentified FL peaks) released from alkaline hydrolysis of STR. (D) Hydroxycinnamic acid derivatives and flavonoids released from alkaline hydrolysis of the trichome STR. Absorbance axes

are scaled to include the largest peak in each chromatogram and are not quantitatively comparable between samples.

Figure 6. Contact angles of intact peach surfaces and water (A), glycerol (C) and diiodomethane (E), and shaved peach surfaces and water (B), glycerol (D) and diiodomethane (F).

Figure 7. Water loss of intact versus de-waxes and shaved peaches (2 days at 24ºC and 40% RH). Data are means ± SD.



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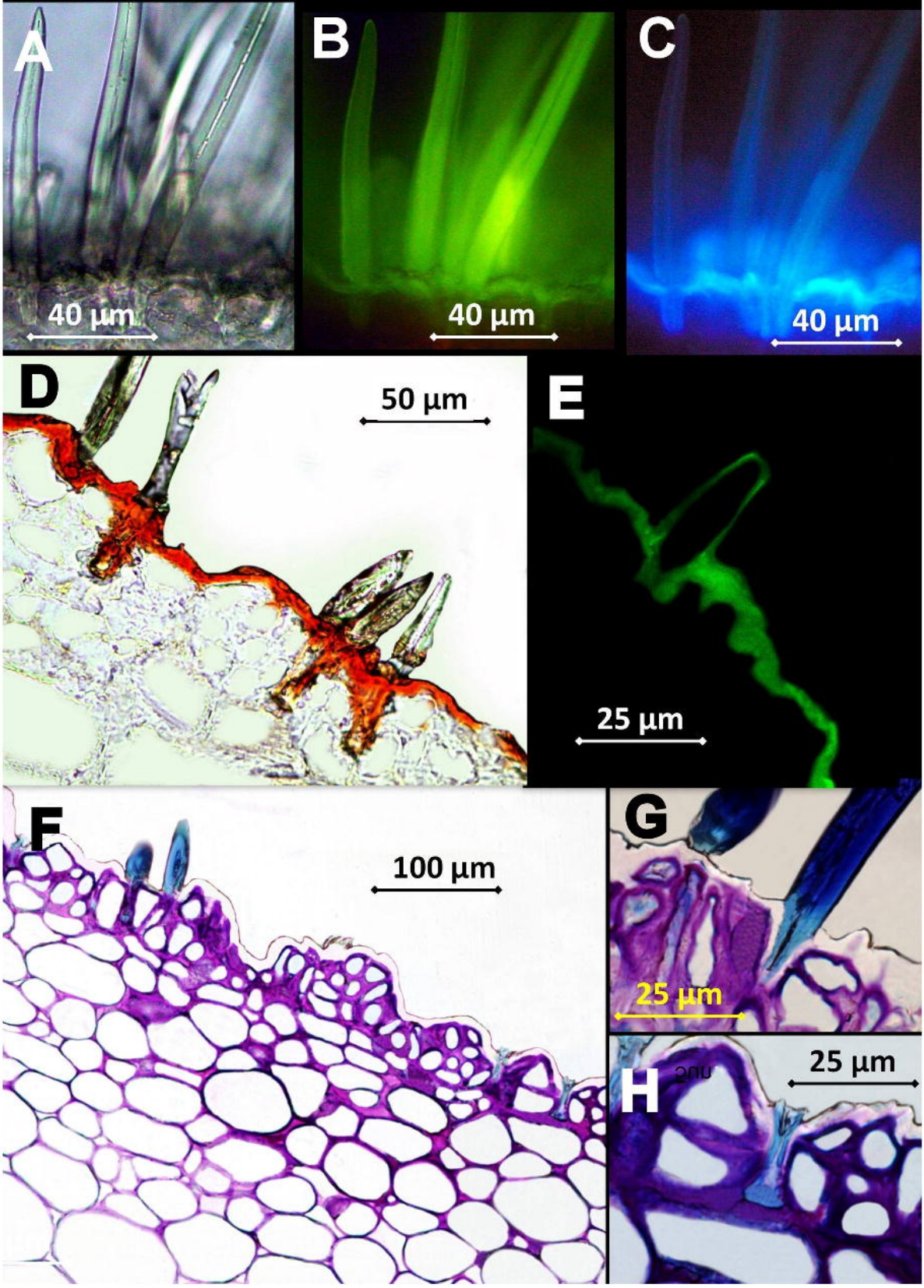

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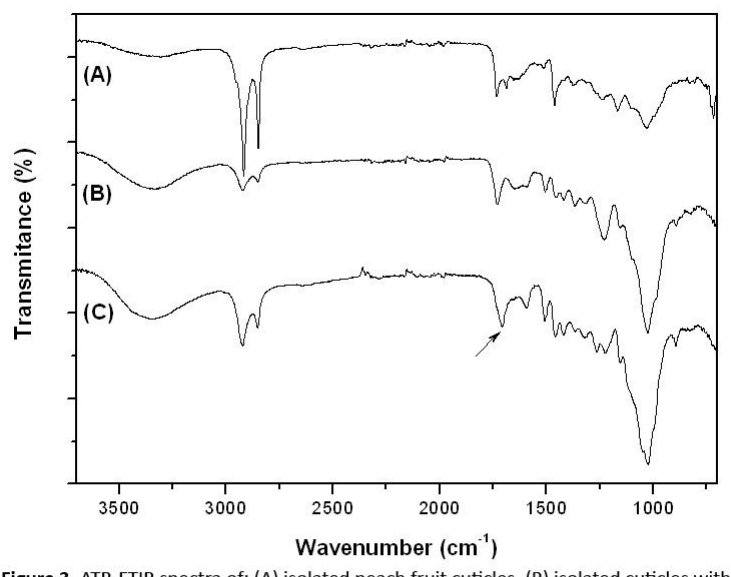

Figure 3. ATR-FTIR spectra of: (A) isolated peach fruit cuticles, (B) isolated cuticles without waxes, and (C) residue after chemical depolymerisation of cutin. Spectra (B) and (C) show significant losses of aliphatic components and a higher presence of polysaccharides. The arrow in (C) indicates a shift from ester to carboxylate groups, indicating the presence of cutan.

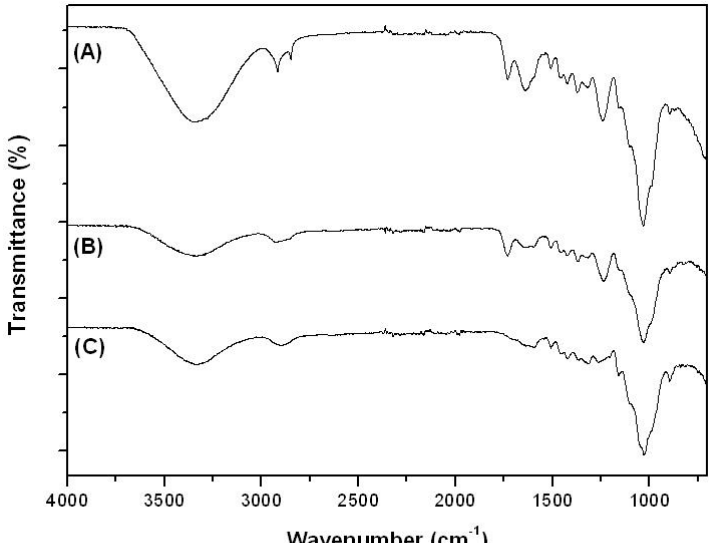

Figure 4. ATR-FTIR spectra of: (A) isolated peach fruit trichomes, (B) isolated trichomes without waxes, and (C) residue after depolymerisation of cutin. The overall spectra have a strong polysaccharide character, typical of cell wall isolates.

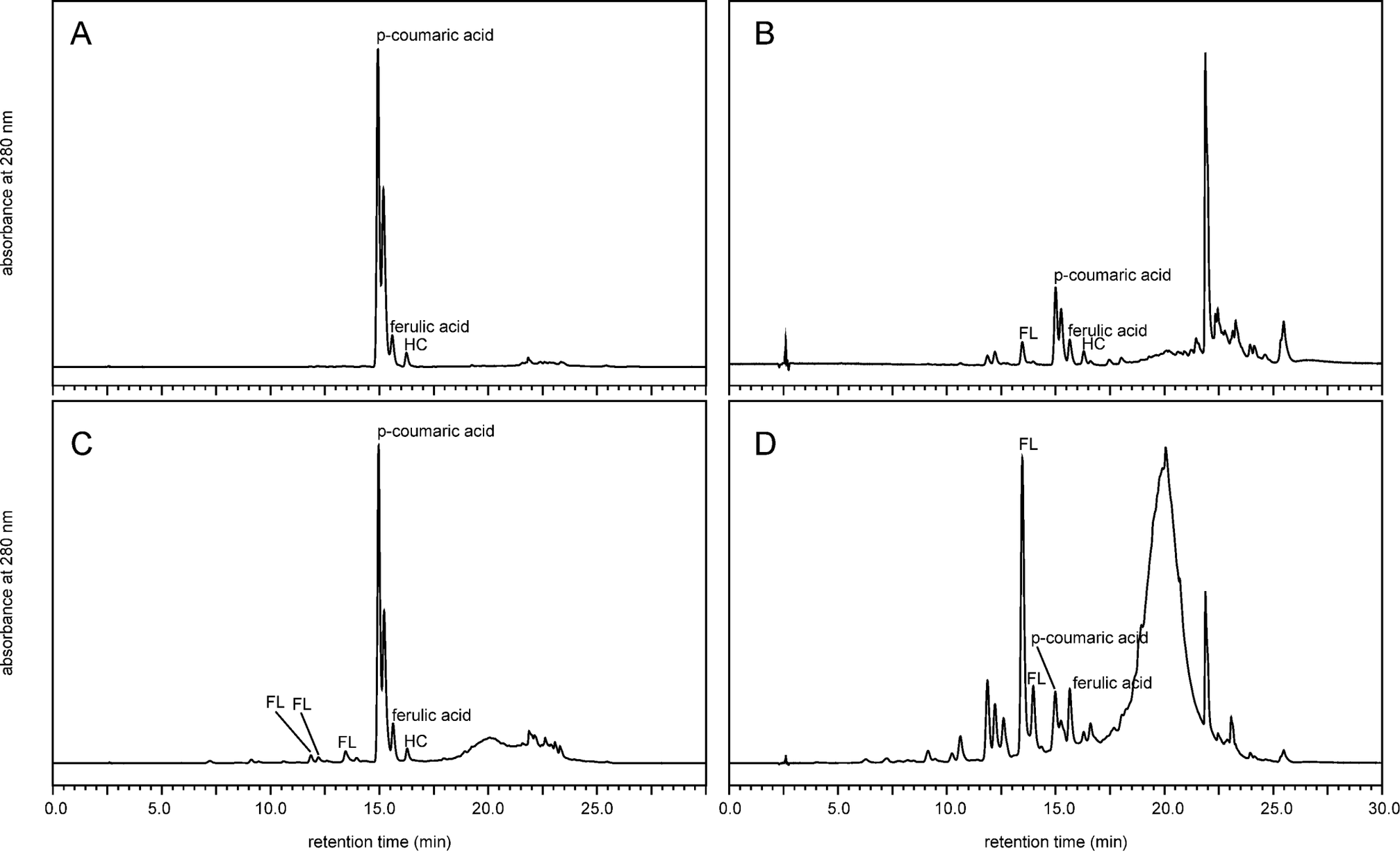

Figure 5. Chromatograms of (A) Hydroxycinnamic acid derivatives (*p*-coumaric acid, ferulic acid and unidentified HC peak) released from alkaline hydrolysis of chloroform isolated cuticular waxes. (B) Hydroxycinnamic acid derivatives and flavonoid (unidentified FL peak) released from alkaline hydrolysis of chloroform isolated trichome waxes. (C) Hydroxycinnamic acid derivatives and flavonoids (unidentified FL peaks) released from alkaline hydrolysis of STR. (D) Hydroxycinnamic acid derivatives and flavonoids released from alkaline hydrolysis of the trichome STR. Absorbance axes are scaled to include the largest peak in each chromatogram and are not quantitatively comparable between samples.

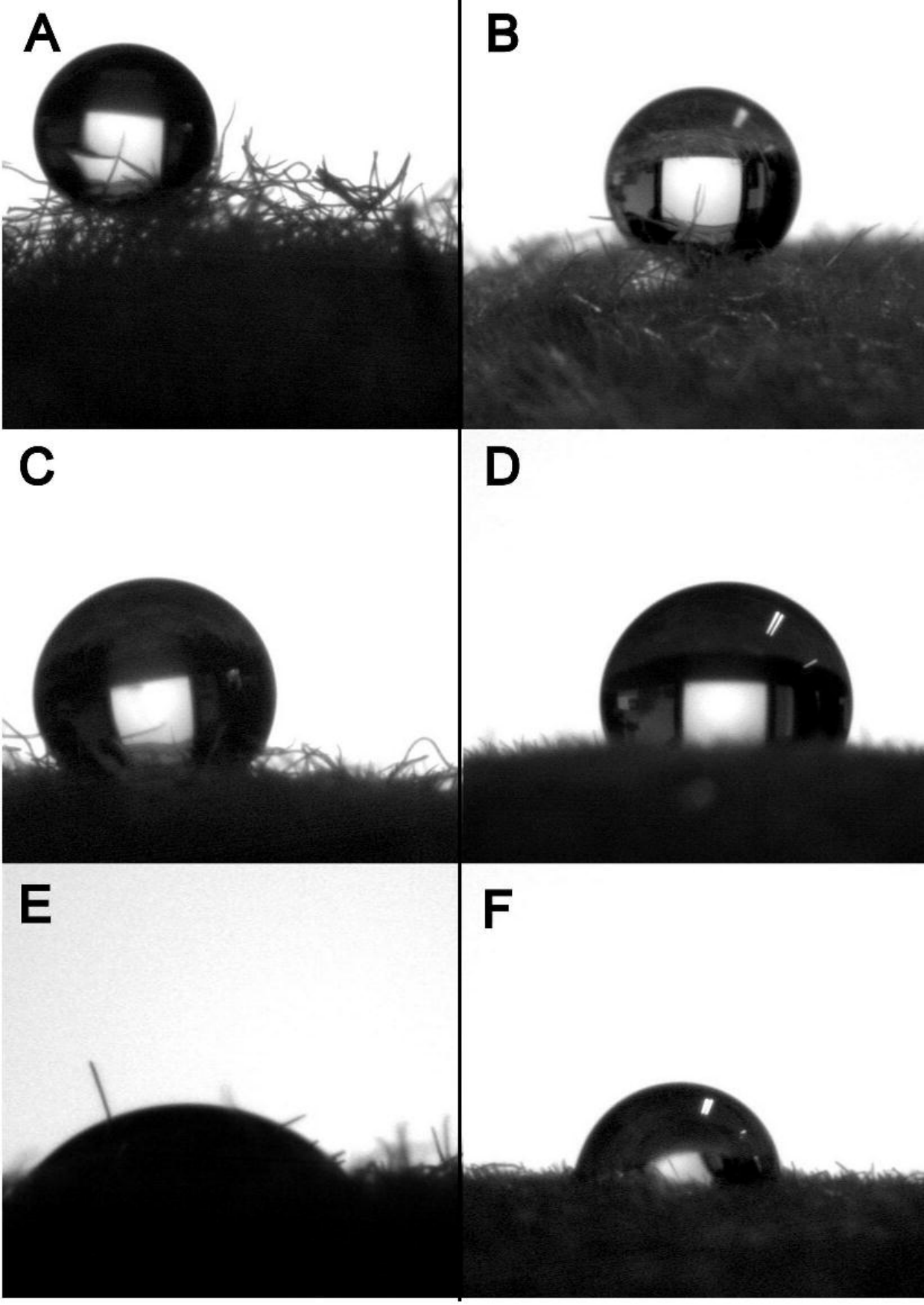

Figure 6. Contact angles of intact peach surfaces and water (A), glycerol (C) and diiodomethane (E) and shaved peach surfaces and water (B), glycerol (D) and iiodomethane (F)

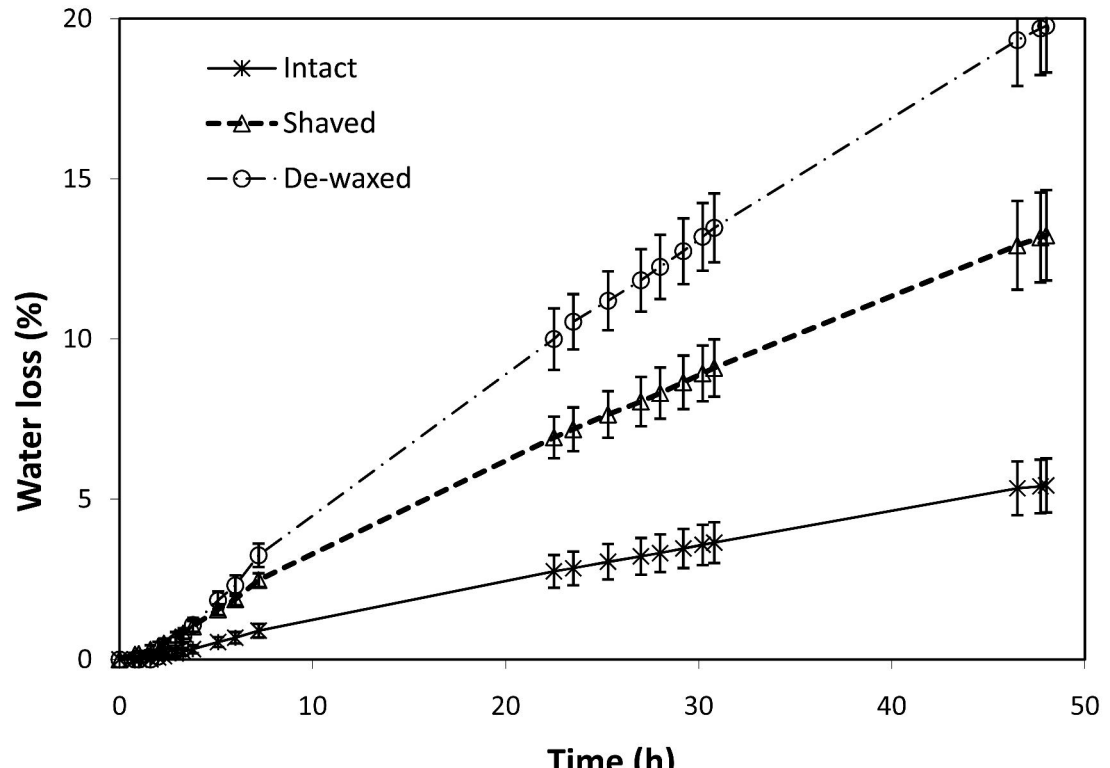

Figure 7. Water loss of intact versus de-waxes and shaved peaches (2 days at 24ºC and 40% RH). Data are means ± SD.