TOTAL WAX AND *N*-ALKANE PROFILES FROM FRUIT AND LEAF WAXES OF *MALPIGHIA GLABRA* L.

DÉBORAH YARA ALVES CURSINO DOS SANTOS* &
ANARY PRISCILA MONTEIRO EGYDIO

Departamento de Botânica, Instituto de Biociências, Universidade de São Paulo,
Rua do Matão, 277, 05508-090 - São Paulo, SP, Brazil.
*Autor para correspondência: dyacsan@ib.usp.br.

Abstract - (Total wax and *n*-alkane profiles from fruit and leaf waxes of *Malpighia glabra* L.) - *Malpighia glabra* L., popularly known as "acerola" or Barbados cherry, is an economically important species due the high levels of ascorbic acid (vitamin C) in its fruits. Cuticular wax affects post-harvest storage. Nevertheless, no data are available for wax composition on this species. Crude wax amount and *n*-alkane profiles have been evaluated for three distinct genotypes of acerola. The total amount of wax ranged from 11 $\mu g.cm^{-2}$ to 24 $\mu g.cm^{-2}$ on leaves and 10 $\mu g.cm^{-2}$ to 30 $\mu g.cm^{-2}$ on fruits. Two of the three genotypes presented statistically distinct totals for leaf and fruit-wax. Type A (yellow epicarp) presented the highest amounts of crude wax on both leaves and fruits. *n*-Alkanes ranged from C_{18} to C_{34} in fruits, with *n*-pentacosane (C_{25}) as the main homologue of most individuals. Narrower *n*-alkane distribution was found in cuticular foliar wax, with C_{22}-C_{33}, with *n*-hentriacontane (C_{31}) as the major component of most individuals. The environmental and economical aspects related to total wax amounts and *n*-alkane profiles are discussed herein.

Resumo - (Cera total e perfil dos *n*-alcanos de frutos e folhas de *Malpighia glabra* L.) - *Malpighia glabra* L., conhecida popularmente como acerola, é uma espécie economicamente importante devido ao elevado nível de ácido ascórbico (vitamina C) em seus frutos. A cera cuticular afeta o armazenamento do fruto pós-colheita. No entanto, não há dados disponíveis sobre a composição das ceras nessa espécie. Teor de cera total e perfil de *n*-alcanos foram estudados em três genótipos diferentes de acerola. A quantidade de cera total variou de 11 $\mu g.cm^{-2}$ a 24 $\mu g.cm^{-2}$ nas folhas e nos frutos de 10 $\mu g.cm^{-2}$ a 30 $\mu g.cm^{-2}$. Dois dos três genótipos apresentaram diferenças significativas entre os teores de cera dos frutos e das folhas. O tipo A (epicarpo amarelo) apresentou a maior quantidade de cera tanto nas folhas como nos frutos. O perfil de *n*-alcanos nos frutos variou de C_{18}-C_{34}, sendo *n*-pentacosano (C_{25}) o homólogo principal na maioria dos indivíduos. A cera cuticular foliar apresentou um perfil de *n*-alcanos com distribuição mais restrita, com os homólogos C_{22}-C_{33} como componentes majoritários. Os homólogos de cadeia curta foram detectados sempre em quantidades menores do que 1%. O *n*-hentriacontano (C_{31}) foi o *n*-alcano principal na cera foliar. Implicações econômicas e ecológicas relacionadas à quantidade das ceras cuticulares e ao perfil de *n*-alcanos são discutidas.

Key words: *Malpighia glabra*, fruit cuticular wax, leaf cuticular wax, *n*-alkanes

Introduction

The appearance of a cuticular layer covering all primary aerial surfaces of plants is pointed out as one of the most important factors for the establishment of terrestrial plants (Baker 1982). The cuticle is composed of a cutin matrix which is either embedded (intracuticular) or covered (epicuticular) by wax. The cutin matrix consists of a polymer typically composed of esterified hydroxyl- and polyhydroxy derivatives from C_{16} and C_{18} fatty acids. When considering the isolated cuticle mass range from 2000 $\mu g.cm^{-2}$ (in fruits) to 450-800 $\mu g.cm^{-2}$ (in leaves), and assuming that 40 – 80 % corresponds to the cutin matrix, this layer can be assumed to be an important plant polymer (Heredia 2003). Waxes are complex mixtures of long-chain aliphatic homologous series (e.g *n*-alkanes, alcohols, aldehydes, fatty acids, and esters), with minor amounts of terpenoids and cinamic acid derivatives. An additional non-hydrolysable fraction of the cuticular membrane, named cutan, is poorly understood as to its structure and chemical composition (Villena et al. 1999, Heredia 2003).

Since the cuticle is the outermost layer of aerial organs, several functions have been associated to this layer, such as being an efficient barrier against excessive water loss, in the control of dust and/or spores adhesion over the plant surface, as a plant-herbivory interaction mediator and, more recently, it has been demonstrated to be crucial in ensuring organ identity during development by preventing the fusion of cell walls from adjacent organs (Yephremov et al. 1999, Sieber et al. 2000, Wellesen et al. 2001 & Jenks et al. 2002).

The association of increased wax-layers with drought stress has been the subject of several investigations. Bondada

et al. (1996) observed an increase in the amount of wax on both leaves and bracts, respectively, from 91.7 $\mu g.cm^{-2}$ to 154.6 $\mu g.cm^{-2}$ and from 74.2 $\mu g.cm^{-2}$ to 108.9 $\mu g.cm^{-2}$, when cotton plants were submitted to a reduction in water.

Cuticular wax plays a crucial role in limiting transpiration across the plant surface. Wax amount and composition have been related to fruit dehydration sensibility (Riederer & Schreider 2001, Smith et al. 2006), which emphasizes its important role in the fruit market. For apples, the protective function of wax as a water-loss barrier is not only important while fruit is still on the tree. During a long storage period, commonly applied to this fruit, wax integrity guarantees both fruit quality and appearance (Veraverbeke et al. 2001). Vogg et al. (2004), on analyzing lines of mutant tomato, concluded that wax composition has an important bearing on cuticular transpiration. Among wax compounds, *n*-alkanes play a critical role in the efficiency of this layer against water loss in both fruits (Sala 2000, Veraverbeke et al. 2001), and leaves (Oliveira et al. 2003). However, there is no consensus regarding the correlation between wax-amount/chemical composition and water barrier effectiveness.

Malpighia glabra L., popularly known as the West Indian cherry, Barbados cherry, and acerola, is an important economical species due to its remarkable ascorbic acid (vitamin C) concentrations in fruits. Although acerola originated in Central America, it has been widely cultivated in Brazil and other tropical countries. This fruit was first introduced into Brazil in the 1950s, and was initially propagated from seeds, which incentivized high genetic variability among fruits from native plantations (Rosso & Mercadante 2005). By 1996, the Brazilian area under cultivation reached 7000 ha, with a yield of more than 32,000 tons (Pertinari & Tarsitano 2002), consumed *in natura* or frozen (whole or isolated pulp) (Oliveira & Filho 1998).

To date there is no information available on the amount of wax and its composition in *Malpighia* species. The present investigation evaluates total cuticular wax yield and the *n*-alkane profile of three distinct genotypes of acerola harvested in a southeastern Brazilian plantation and suggests that this differences could affect post-harvest shelf life.

Material and Methods

Fruit and leaf samples were harvested in a small farm (Sítio Sete Irmãos) situated in Indaiatuba (São Paulo State - Brazil) (23º05'25"S and 47º13'05"W). Total farm-area presented around 4000 plants with high phenotypic variability. Twenty-four individuals representing three distinct genotypes that were selected, based mainly on the color of their epicarp: type A (yellow epicarp – 4 individuals), type B (light-red epicarp – 10 individuals), and type C (deep-red epicarp – 10 individuals).

Surface fruit area was calculated based on an elliptic surface area formula, and for leaf area the Skye Instrument was employed with Leaf Measurement System v.2.0 software.

Total cuticular wax was extracted by dipping the samples three times (30 sec. each) in chloroform. The extracts were pooled together and evaporated to dryness in a rotative evaporator under reduced pressure. Each individual sample corresponds to pools of 30 fruits and 30 leaves, randomly picked from trees. The amount of wax is calculated as $\mu g.cm^{-2}$.

n-Alkanes were isolated by silica (70-230 mesh - Merck) column chromatography eluted with *n*-hexane, and homologous series identified by GC/MS (HP 5890 ser. II plus/HP 5989B), applying the following conditions: source temperature 200ºC, quadrupole temperature 100ºC, EM 70eV, capillary column DB 5HT of 30m x 0.32mm i.d. (J&W Scientific), injector and detector temperatures 300ºC, helium as carrier-gas at a flow rate of 1 $mL.min^{-1}$. Oven-heat program: initial temperature 140ºC held for 1 min, increased by $10ºC.min^{-1}$, until the final temperature of 300ºC remained unchanged for 10min.

Quantitative wax data were analyzed by One Way ANOVA. Statistically distinct values ($p \leq 0.05$) were compared by Bonferroni's test using Jandel (SigmaStat) software (Neter et al. 1996).

Results and Discussion

The total amount and composition of fruit and of leaf waxes are susceptible to certain biotic and abiotic factors, such as geographic location, light intensity, humidity, plant species and age and harvested organ (Baker 1982).

Table 1 shows total amounts of cuticular wax ($\mu g.cm^{-2}$) on fruits and leaves from three acerola genotypes. Types A (yellow epicarp) and C (deep-red epicarp) presented significant differences ($p \leq 0.05$) between total wax on both fruits and leaves, which was not observed for type B. Available data were not sufficient to define which plant organ presented the thickest layer of wax. Whereas type A presented, on average, higher total amounts of wax on fruits (26 $\mu g.cm^{-2}$) than on leaves (21 $\mu g.cm^{-2}$), type C showed the opposite. The average of total cuticular wax for leaves of type C was higher (16.7 $\mu g.cm^{-2}$) in comparison to fruit-layers (14.3 $\mu g.cm^{-2}$). Baker (1982) has suggested that layers of cuticular wax on fruits are always thicker than those on leaves in the same species. Rosenquist and Morrison (1989) observed thicker cuticular layers on grape fruits than on leaves. Values for acerola type C and type B do not confirm these suggestions. Type C presented higher values on leaf than on fruit surfaces, while no significant difference was found for type B.

Distinct varieties of one crop may present different amounts of cuticular wax. A comparison among the three acerola genotypes unveils type A with higher values for both fruit and leaf cuticular wax than types B and C. Sala et al. (1992) detected different amounts of cuticular wax on fruit surfaces of several *Citrus* sp cultivars. Quantitative variation in total wax and isolated compounds was also detected in apples (Verabereke et al. 2001), potatoes (Szafranek & Synak 2006), sesames (Kim et al. 2007a), and soybeans (Kim et al. 2007b).

Although acerola has been widely cultivated in Brazil and others countries, there are no consensual definitions regarding cultivars and/or the establishment of varieties. Oliveira and Filho (1998) suggested that color of epicarp, which ranges from yellow to dark-red, is a valuable parameter in putative cultivar delimitations. Light colored fruits (type A), e.g. non-red epicarp, presented a larger wax-layer. However, other intermediate fruit-color genotypes should be investigated to confirm this hypothesis.

Post-harvest water loss by fruits results in fruit softening besides reduced glossiness and shelf life (Smith et al. 2006). Since plant cuticle and consequently cuticular wax act as a water-loss barrier, any information related to this layer may be useful to address differences in this physiological trait. Albrigo (1972) encountered a negative correlation between wax-amount and water loss in postharvest fruits. Methods and periods of harvesting and storage may affect the amount of wax and composition, whereby this may have an influence on the quality of the fruit.

The acerola market is variable. Whereas for the Brazilian market preference is for deep-red fruits, European consumers valorize fruits with a light-colored epicarp, these being used for vitamin C enrichment of other fruit juices (Gonzaga Neto et al. 1998). Light-colored fruit trees are rare in Brazilian crop areas, a reason why a small number of samples were accessible for this genotype. Total fruit wax in type A, which was significantly ($p \leq 0.05$) higher than in the other two analyzed types (Table 1), may be considered an important and valuable characteristic, since it can increase fruit resistance and consequently improve exportation of these fruits.

Another important factor worthy of note is that the amount of cuticular wax is not always inversely correlated with transpiration rate or positively with drought-tolerance (Ristic &Jenks 2002). Previous papers suggest that wax composition, rather than total wax layer, is more effective as a barrier for non-stomatic water loss. Aliphatic long chain *n*-alkanes from apple (Veraverbeke et al. 2001) and tomato (Vogg et al. 2004) fruit waxes have a greater influence on post-harvest fruit appearance than other wax components.

n-Alkanes composition was determined by GC/MS for leaf and epicarp waxes of acerola (Tables 2 and 3). Fruit *n*-alkane profiles were more variable than those of leaves, ranging from C_{18} to C_{34}. Most analyzed samples presented *n*-pentacosane (C_{25}) as the major constituent in the fruit *n*-alkane fraction, followed by *n*-heptacosane (C_{27}) and *n*-nonacosane (C_{29}). Those samples which did not present C_{25} as the main homologue have been characterized by C_{29} as the major *n*-alkane component, followed by similar percentages of C_{25} and C_{27}. *n*-Nonacosane, as the main component of the *n*-alkane fraction of fruit cuticular waxes, has already

Table 1. Total cuticular wax (μg.cm^{-2}) of fruits and leaves of three acerola genotypes.

	Genotypes					
	A		B		C	
Samples	FRUIT	LEAF	FRUIT	LEAF	FRUIT	LEAF
1	26	20	10	15	17	18
2	30	24	11	18	12	14
3	22	20	12	14	16	19
4	26	20	12	17	17	16
5			13	15	13	18
6			15	17	16	17
7			16	11	12	19
8			20	11	12	14
9			21	18	13	14
10			14	17	15	18
means ± SD*	26±3.27Aa	21±2.0Ba	14.4±3.69Ab	15.3±2.63Ab	14.3±2.11Bb	16.7±2.06Ab

Each individual sample corresponds to 30 fruits and/or leaves harvested from the same tree.
Genotype A: yellow epicarp, genotype B: light-red epicarp, genotype C: deep-red epicarp.
* Numbers followed by distinct letters indicate significant differences ($p \leq 0.05$). Capital letters compare total fruit and leaf-wax values of each type. Lowercase letters compare total fruit or leaf-wax among the three acerola types.

been described for sweet cherry - *Prunus avium* (Peschel et al. 2007) and apple - *Malus domestica* (Belding et al. 1998, Veraverbeke et al. 2001).

The detection of C_{25} as the main homologue in fruit cuticular waxes seems to be unusual among several of the investigated species. Short/medium-chain length alkanes in fruit waxes (C_{25} – C_{27}) have been suggested as essential compounds in chilling-injury protection. Changes in these homologue concentrations were correlated to damage reduction caused by low temperature storage (Nordby & McDonald 1991). The ratio of short/medium-chain length (C_{19} - C_{27}) over long-chain length (C_{29} - C_{34}) alkanes among acerola fruits was generally 2:1. Distinct ratio values were found only in those samples with C_{29} as the main homologue

Table 2. *n*-Alkane profiles of fruit cuticular wax from three acerola genotypes. Values are given as percentages (-: non-detected homologue, t: values lower than 1%).

Samples*		*n*-alkane chain length																
		C18	C19	C20	C21	C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33	C34
A	1	4	2	t	t	t	7	6	33	8	22	3	9	6	-	-	-	-
	2	t	t	t	t	t	3	3	6	8	11	14	16	11	11	10	4	3
	3	-	t	t	t	t	3	6	19	6	15	3	23	2	21	t	2	-
	4	12	31	t	t	t	t	t	57	t	t	t	t	t	t	-	-	-
B	1	5	2	4	2	4	5	9	13	12	11	7	18	4	4	-	-	-
	2	t	4	6	5	8	15	10	8	8	8	6	9	4	5	4	-	-
	3	2	1	3	1	7	10	19	16	16	11	6	6	1	1	-	-	-
	4	t	t	2	2	3	8	1	20	16	17	11	12	4	4	-	-	-
	5	7	t	5	t	5	6	11	17	13	8	7	7	8	6	t	-	-
	6	2	t	8	3	9	11	14	16	14	10	6	7	t	t	-	-	-
	7	-	t	t	t	t	8	13	31	13	15	9	11	t	t	t	-	-
	8	2	2	2	4	6	11	12	15	13	10	8	7	4	4	-	-	-
	9	t	-	10	t	8	20	13	38	4	7	t	t	t	t	t	-	-
	10	t	t	t	t	t	8	8	18	14	17	13	14	8	t	t	-	-
C	1	3	9	t	t	t	20	t	30	1	13	t	24	-	t	-	-	-
	2	12	20	t	t	t	5	7	45	6	5	t	t	t	t	-	-	-
	3	-	t	t	t	t	17	t	62	t	t	t	21	-	-	-	-	-
	4	3	4	6	4	6	8	12	22	14	12	5	4	t	-	-	-	-
	5	2	2	2	4	6	11	12	15	13	10	8	7	4	4	-	-	-
	6	-	-	-	-	-	t	7	26	-	24	6	37	-	-	-	-	-
	7	-	t	t	t	t	11	5	19	7	21	6	23	t	8	-	-	-
	8	4	16	t	t	t	5	2	8	2	11	t	44	-	8	-	-	-
	9	-	-	-	-	-	t	7	26	-	24	6	37	-	-	-	-	-
	10	-	t	t	t	t	11	5	19	7	21	6	23	t	8	-	-	-

* Letters correspond to acerola genotypes (A: yellow epicarp, B: light-red epicarp, C: deep-red epicarp). Numbers correspond to each individual sample of 30 fruits harvested from the same tree.

(Table 2). The high proportion of short/medium-chain length *n*-alkanes in these fruits may contribute to their long-term low temperature storage. However, detailed investigations on storage time, temperature and wax composition relationships are needed for this species.

Similar to fruits, total cuticular leaf-wax did not present wide variation (Table 1). Table 3 reveals *n*-alkane profiles for leaf-wax samples consisting of odd and even carbon-numbered molecules from C_{22} – C_{33}, with *n*-hentriacosane (C_{31}) as the main homologue, followed by C_{29}. Variations in these profiles were much lower in leaf-wax in comparison to those observed in fruits. Long chain *n*-alkanes, mainly C_{31}, are also major constituents in the leaves of several important crops, such as sesame (Kim et al. 2007a), soybean (Kim et al. 2007b), potato (Szafranek & Synak 2006), tomato (Smith et al. 2006) and cotton (Bondada et al. 1996).

Odd carbon-numbered chain *n*-alkanes dominated in fruit and leaf waxes of most individuals, except for two samples of type B (respectively, individual 3 – Table 2 and individual 2 – Table 3). In higher plant waxes, odd carbon-numbered *n*-alkanes predominate over even carbon-numbered molecules. Even carbon-numbered *n*-alkane biosynthesis is not completely understood (Kunst & Samuels 2003). The widespread occurrence of even carbon-numbered alkanes

Table 3. *n*-Alkane profiles of leaf cuticular wax from three acerola genotypes. Values are given as percentages. (-: non-detected homologue, t: values lower than 1%).

Samples*		*n*-alkane chain length											
		C22	C23	C24	C25	C26	C27	C28	C29	C30	C31	C32	C33
A	1	t	2	4	8	4	16	4	22	4	27	3	6
	2	t	t	t	t	t	12	5	15	8	43	6	11
	3	t	t	t	t	t	13	t	32	9	36	t	10
	4	t	t	t	t	t	10	t	16	8	39	13	14
B	1	t	t	t	t	t	5	t	15	6	52	10	12
	2	t	t	t	t	t	t	t	19	t	23	58	t
	3	-	-	-	-	-	t	t	11	t	64	t	25
	4	1	3	4	5	3	15	5	26	8	22	4	4
	5	-	-	t	t	t	9	3	17	6	38	8	19
	6	t	t	t	t	t	8	t	29	t	51	t	12
	7	t	t	1	2	2	10	4	27	4	37	5	8
	8	t	t	t	t	t	7	t	14	6	51	10	12
	9	t	t	t	t	t	11	4	24	8	37	7	9
	10	t	t	t	3	3	10	5	32	5	31	5	6
C	1	t	t	7	13	8	13	4	23	t	32	t	t
	2	t	t	t	t	t	22	t	26	t	52	t	t
	3	t	t	t	t	t	10	t	45	t	45	t	t
	4	t	t	t	t	-	t	t	37	t	63	-	-
	5	-	-	t	t	t	4	-	28	6	45	3	14
	6	t	t	t	t	t	11	t	23	t	47	t	19
	7	-	t	t	t	t	12	t	33	t	38	t	17
	8	3	t	t	t	t	6	4	15	5	42	11	14
	9	t	t	t	t	t	11	4	24	8	37	7	9
	10	t	t	t	t	t	t	t	29	t	53	t	18

* Letters correspond to acerola genotypes (A: yellow epicarp, B: light-red epicarp, C: deep-red epicarp). Numbers correspond to each individual sample of 30 leaves harvested from the same tree.

may offer an interesting material for biosynthesis studies of these compounds.

Long chain *n*-alkanes have markedly been suggested as ubiquitous components in drought-adapted species. Oliveira et al. (2003) pointed out that thicker leaf-wax layers did not reduce water loss. However, *n*-alkanes ranging from C_{27} to C_{33} were two or three-fold more efficient than triterpenes as a transpiration barrier.

Water-deficiency may negatively affect several physiological processes in a wide range of crops. Increased leaf-wax layers have already been reported as being adaptive answers of certain crops to water-stress conditions. Nevertheless, recent research using sesame (Kim et al. 2007a) and soybean (Kim et al. 2007b) have demonstrated that cultivars with more drought-induced wax (a condition that should improve plant vigor under drought) produced fewer seeds after drought-stress, an unexpected response. These authors suggested that the most drought-tolerant cultivars could/may abort some of their seeds, and more efficiently allocate resources for leaf-wax synthesis, so as to improve water retention and assure survival of the mother-plant. This process would promote healthy growth of the remaining seeds, and maintain a more vigorous mother-plant. After drought has been alleviated, the plant is able to generate more new inflorescences (and seeds) than the less drought-responsive plants.

Complete knowledge regarding cuticular wax and its relationships with external biotic and abiotic factors requires intense research efforts. Elucidation of these interactions will certainly generate consequences for crop production, as well as for the improvement of post-harvest storage conditions.

This is the first report on cuticular wax-yield and partial composition in *Malpighia glabra* (acerola), and suggests significant differences in fruit and leaf cuticular wax amount and composition among distinct genotypes. Further studies on complete wax composition and its role in post-harvest fruit storage are needed in order to improve available data for the economic use of this species.

Acknowledgements

The authors would like to thank CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior) for the Egydio, A.P.M. fellowship, as well as FAPESP (Fundação de Amparo à Pesquisa do Estado de São Paulo) and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) for financial support. We are especially grateful to Mr. Ude Tomazeto for permission to harvest.

References

ALBRIGO, L.G. 1972. Ultrastructure of cuticular surfaces and stomata of developing leaves and fruit of the Valencia oranges. *J. Am. Soc. Hortic. Sci.* 97:761-765.

BAKER, E.A. 1982. Chemistry and morphology of plant epicuticular waxes. In D.F. Cutler, K.L. Alvin & C.E. Price (eds) *The Plant Cuticle.* Academic Press, London, p. 139-165.

BELDING, R.D., BLANKENSHIP, S.M., YOUNG, E. & LEIDY, R.B. 1998. Composition and variability of epicuticular waxes in apple cultivars. *J. Am. Soc. Hortic. Sci.* 123: 348-356.

BONDADA, B.R., OOSTERHUIS, D.M., MURPHY, J.B. & KIM, K.S. 1996. Effect of water stress on the epicuticular wax composition and ultrastructure of cotton (*Gossipium hirsutum* L.) leaf, bract, and boll. *Environm. Exp. Bot.* 36: 61-69.

GONZAGA NETO, L., MATTUZ, B.H. & SANTOS, C.A.F. 1998. Caracterização agronômica de clones de aceroleira (*Malpighia* spp) na região do submédio São Francisco. *Revista Bras. Frutic.* 21:110-115.

HEREDIA, A. 2003. Biophysical and biochemical characteristics of cutin, a plant barrier biopolymer. *Biochem. Biophys. Acta* 1620: 1-7.

JENKS, M.A., EIGENBRODE, S.D. & LEMIEUX, B. 2002. Cuticular waxes of *Arabidopsis*. In C.R. Somerville and E.M. Meyerowitz (eds) *The Arabidopsis Book.* American Society of Plant Biologists, Rockville. pp. 1-22.

KIM, S.K, PARK, S.H. & JENKS, M.A. 2007a. Changes in leaf cuticular waxes of sesame (*Sesamum indicum* L.) plants exposed to water deficit. *J. Plant Physiol.* 164: 1134-1143.

KIM, S.K, PARK, S.H., KIM, D.K. & JENKS, M.A. 2007b. Influence of water deficit on leaf cuticular waxes of soybean (*Glycine max* [L.] Merr.). *Int. J. Pl. Sci.* 168: 307-316.

KUNST, L. & SAMUELS, A.L. 2003. Biosynthesis and secretion of plant cuticular wax. *Prog. Lipid Res.* 42: 51-80.

NETER, J., KUTNER, M.H., NACHTSHEIM, C.J. & WASSERMAN, W. 1996. *Applied linear statistical models*. Truvim, Chicago.

NORDBY, H.E. & MCDONALD, R.E. 1991. Relationship of epicuticular wax composition of grapefruit to chilling injury. *J. Agric. Food Chem.* 39: 957-962.

OLIVEIRA, A.F.M., MEIRELLES, S.T. & SALATINO, A. 2003. Epicuticular waxes from caatinga and cerrado species and their efficiency against water loss. *Anais Acad. Brasil. Cienc.* 75: 431-439.

OLIVEIRA, J.R.P. & FILHO, W.S.S. 1998. Situação da cultura da acerola no Brasil e ações da Embrapa Mandioca e Fruticultura em recursos genéticos e melhoramento. Recursos Genéticos e Melhoramento de plantas para o Nordeste Brasileiro. *www.cpatsa.embrapa.br/catalogo/livrorg/acerolabrasil.pdf* (accessed 15 December 2009).

PESCHEL, S., FRANKE, R., SCHREIBER. L. & KNOCHE, M. 2007. Composition of the cuticle of developing sweet cherry fruit. *Phytochemistry* 68: 1017–1025.

PETINARI, R.A. & TARSITANO, M.A.A. 2002. Análise econômica da produção de acerola para mesa em Jales-SP: um estudo de caso. *Revista Bras. Frutic.* 24: 411-415.

RIEDERER, M. & SCHREIBER, L. 2001. Protecting against water loss: analysis of the barrier properties of plant cuticles. *J. Exp. Bot.* 52: 2023-2032.

RISTIC, Z. & JENKS, M.A. 2002. Leaf cuticle and water loss in maize lines differing in dehydration avoidance. *J. Plant Physiol.* 159: 645–651.

ROSENQUIST, J.K. & MORRISON, J.C. 1989. Some factors affecting cuticle and wax accumulation on grape berries. *Amer. J. Enol. Vitic.* 40: 241-244.

ROSSO, V.V. & MERCADANTE, A.Z. 2005. Carotenoid composition of two Brazilian genotypes of acerola (*Malpighia punicifolia* L.) from two harvests. *Food Res. Int.* 38: 1073–1077.

SALA, J.M. 2000. Content, chemical composition and morphology of epicuticular wax of fortune mandarin fruits in relation to peel pitting. *J. Sci. Food Agric.* 80: 1887-1894.

SALA, J.M., LAFUENTE, T. & CUNA, T.P. 1992. Content and chemical-composition of epicuticular wax of navelina oranges and satsuma mandarins as related to rindstaining of fruit. *J. Sci. Food Agric.* 59: 489-495.

SIEBER, P., SCHORDERET, M., RYSER, U., BUCHALA, A., KOLATTUKUDY, P., MÉTRAUX, J.-P. & NAWRATH, C. 2000. Transgenic *Arabidopsis* plants expressing a fungal cutinase show alterations in the structure and properties of the cuticle and postgenital organ fusions. *Pl. Cell* 12: 721-737.

SMITH, D.L., STOMMEL, J.R., FUNG, R.W.M., WANG, C.Y. & WHITAKER, B.D. 2006. Influence of cultivar and harvest method on postharvest storage quality of pepper (*Capsicum annuum* L.) fruit. *Postharv. Biol. Technol.* 42: 243-247.

SZAFRANEK, B.M. & SYNAK, E.E. 2006. Cuticular waxes from potato (*Solanum tuberosum*) leaves. *Phytochemistry* 67: 80-90.

VERAVERBEKE, E.A., LAMMERTYN, J., SAEVELS, S. & NICOLAÏ, B.M. 2001. Changes in chemical wax composition of three different apple (*Malus domestica* Borkh) cultivars during storage. *Postharv. Biol. Technol.* 23: 197-208.

VILLENA, J.F., DOMÍGUEZ, E., STEWART, D. & HEREDIA, A. 1999. Characterization and biosynthesis of non-degradable polymers in plant cuticles. *Planta* 208: 181-187.

VOGG, G., FISCHER, S., LEIDE, J., EMMANUEL, E., JETTER, R., LEVY, A.A. & RIEDERER, M. 2004. Tomato fruit cuticular waxes and their effects on transpiration barrier properties: functional characterization of a mutant deficient in a very-long-chain fatty acid β-ketoacyl-CoA synthase. *J. Exp. Bot.* 55: 1401-1410.

WELLESEN, K., DURST, F., PINOT, F., BENVENISTE, I., NETTSHEIM, K., WISMAN, E., STEINER-LANGE, S., SAEDLER, H. & YEPHREMOV, A. 2001. Functional analysis of the *LACERATA* gene of *Arabidopsis* provides evidence for different roles of fatty acid ω-hydroxylation in development. *PNAS* 98: 9694-9699.

YEPHREMOV, A., WISMAN, E., HUIJSER, P., HUIJSER, C., WELLESEN, K. & SAEDLER, H. 1999. Characterization of the *FIDDLEHEAD* gene of *Arabidopsis* reveals a link between adhesion response and cell differentiation in the epidermis. *Pl. Cell* 11: 2187-2201.