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Structures and Functions of Cuticular Wax in Postharvest Fruit and Its Regulation: A Comprehensive Review with Future Perspectives



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ABSTRACT

Cuticular wax plays a major role in the growth and storage of plant fruits. The cuticular wax coating, which covers the outermost layer of a fruit's epidermal cells, is insoluble in water. Cuticular wax is mainly composed of very long-chain fatty acids (VLCFAs); their derivatives, including esters, primary alcohols, secondary alcohols, aldehydes, and ketones; and triterpenoids. This complex mixture of lipids is probably biosynthesized in the epidermal cells of most plants and exuded onto the surface. Cuticular wax not only makes the fruit less susceptible to microbial infection but also reduces mechanical damage to the fruit, thereby maintaining the fruit's commodity value. To date, research has mostly focused on the changes, function, and regulation of fruit wax before harvest, while ignoring the changes and functions of wax in fruit storage. This paper reviews on the composition, structure, and metabolic regulation of cuticular wax in fruits. It also focuses on postharvest factors affecting wax composition, such as storage temperature, relative humidity (RH), gas atmosphere, and as exogenous hormones; and the effects of wax on fruit postharvest quality, including water dispersion, fruit softening, physiological disorders, and disease resistance. These summaries may be of assistance in better understanding the changes in cuticular wax in postharvest fruit and the resulting effects on fruit quality.

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1. Introduction

The plant cuticle layer is the result of the long-term natural evolution of plants in response to their living environment. It is a natural barrier that protects plants from biological or non-biological stress [1,2]. The cuticle layer is formed by a hydrophobic substance covering the surface of the plant organ or tissue, mainly consisting of cutin and wax. Cutin constitutes the basic frame of the cuticle, which is partially enameled with intracuticular wax, whereas epi-

cuticular wax is distributed outside the cutin frame [3]. Cuticular wax also plays an important role in maintaining fruit quality [4,5]. In recent years, an increased number of studies have focused on cuticular wax in fruit, due to its many physiological functions. The cuticular wax of the fruit epidermis can prevent water loss, nutrient leakage, mechanical damage, and fruit cracking [6–8]. It can also prevent pathogen infection, pest invasion, and damage from external environmental factors such as drought and ultraviolet radiation [9,10]. Fruit cuticular wax is the main barrier preventing water loss and resisting microorganism attacks, and can thereby effectively reduce the phenomenon of fruit shrinkage and quality decline during storage shelf life [11]. Studies on the physiological and biochemical characteristics of cuticular wax after postharvest and its relationship with fruit storage tolerance are

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receiving increasing attention [2,5,12]. Therefore, we summarize here the most updated compilation of cuticular wax structures and functions in the context of postharvest fruit and its regulation. Understanding these issues may provide new ideas and strategies for fruit preservation, with the aim of reducing postharvest losses of fruit.

2. Morphological structure and composition of cuticular wax

2.1. Morphological structure

Cuticular wax consists of intracuticular wax and epicuticular wax [13]. Intracuticular wax usually has an amorphous form, while epicuticular wax is secreted by the cell to the surface of the plant and forms different wax crystals by self-assembling. The diversity of the morphology, size, and distribution of plant wax crystals can be observed via scanning electron microscopy (SEM). Koch and Ensikat [14] reported that the common morphology of epicuticular wax comprises plates and platelets. The main wax morphological types in fruit include amorphous films, plates/platelets, rods/rodlets, and tubes. In addition, minor morphological types such as lamellae and granules/ovate type have been found in fruit (Table 1 [15–32]). The wax morphology in fruits of different species is variable. For example, the cuticular wax in most species of goji berry (*Lycium barbarum* L.) exists as a lamellar structure, but it exists as rodlets and plates in certain cultivars [15]. The forms of wax crystals in different cultivars of pear fruit include amorphous wax, rodlets, platelets, and ovate crystals [16]. Lanza and Di Serio [17] reported that wax morphology in olive peel was mainly granular, platelets, and plates, along with a few rodlets. Chu et al. [18] found that the wax structures of blueberry fruit are mainly rodlets, ranging in length between 2–5 μm .

2.2. Chemical composition

Cuticular wax is mainly composed of very long-chain fatty acids (VLCFAs) and their derivatives (typically between C20–C34), including alkanes, alcohols, esters, aldehydes, and ketones [33]. In addition, special compounds such as triterpenoids are often predominant wax components in certain fruit varieties such as blueberry and bilberry [18,34]. Other compounds that are present at minor levels include sterols and phenols. A list of the composition and content of cuticular wax in fruit reported in the literature is provided in Table 2 [2,4,18,23,26–29,34–51]. The composition and content of the fruit wax of different species differ, as do those between different varieties or cultivars of a species. For example, the total wax contents of different apple cultivars can range from 366.00 to 2185.00 $\mu\text{g}\cdot\text{cm}^{-2}$ [27,35–38]. The difference may be due to different genotypes and growth environments of apples.

2.2.1. VLCFAs and their derivatives

Fatty acids are the precursors of wax synthesis and, as such, are almost always present in the wax of all plants, unlike triterpenoids, which show more specificity in their abundance. Most fatty acids exist in the form of unbranched saturated fatty acids with carbon chains in which the carbon atoms are predominantly even in number (known as an “even-over-odd” predominance), typically ranging from C16 to C34. For example, the waxes in bog bilberry [26], grape [41], and jujube [43] are rich in saturated fatty acids with carbon chains of C16–C32. Hexadecanoic acid (C16:0) and octadecanoic acid (C18:0) are the main fatty acids in many common fruits such as orange [2], apple [37], and tomato [31]. In addition, the waxes of some berry fruits such as blueberry [32] and bayberry (*Myrica pensylvanica*) [52] contain unsaturated fatty acids with an even number of carbon atoms, such as oleic acid (C18:1) and

linoleic acid (C18:2), while dotriacontenoic acid (C32:1) and C30 unsaturated fatty acid are the predominant unsaturated fatty acids in lemon fruit (*Citrus limon*) and orange fruit (*Citrus sinensis*), respectively [25,44].

Alkanes, the most common type of wax component in fruit, are mainly composed of an odd number of carbon atoms. The predominant wax ingredients of sweet orange (*Citrus sinensis* L.) peel are alkanes in the range of C22–C32, among which the proportion of nonacosane (C29) and hentriacontane (C31) are the most abundant [53]. The alkane content in peach fruit wax is second only to the triterpenoid content, accounting for 19% of the total wax, with pentacosane (C25) as the highest alkane content [46]. Alkanes are also the main wax components of lemon, accounting for 50% of the total wax, while in sweet cherry [34], the alkane content is only 0.89%–1.05%.

Alcohols are subdivided into primary alcohols and secondary alcohols, depending on the different position of the hydroxy functional group in the carbon chain skeleton. The hydroxy substituted group in a primary alcohol is located at the terminal position of a hydrocarbon chain. The skin of a blueberry contains 32%–40% primary alcohols, among which C28 alcohol accounts for 81%–87% of the total alcohol content [18]. The presence of primary alcohols has been reported in a variety of plant fruits such as sweet cherry [34], apple [37], and citrus [2]. Secondary alcohols have a hydroxy functional group located in a mid-chain position. The secondary alcohols in apple fruit wax range from C26 to C30 components with odd-numbered carbon chains, and the hydroxy group may be found in positions 8–11 of the straight chain [54].

Secondary alcohols are frequently accompanied by the corresponding ketones, at widely varying concentrations. Ketones account for the second largest fraction in bog bilberry wax (22.50%), but their content is very small in bilberry (3.60%) and crowberry (0.03%) [26]. β -diketones are important components of ketones that are mainly characterized by their number of carbon atoms, which usually range from C27 to C33, as well as the presence of a carbonyl functional group at the C1 and C3 position, respectively. In particular, hentriacontan-10,12-dione and tritriacontan-12,14-dione are present in the cuticular wax of the highbush blueberry and the rabbiteye blueberry, respectively [18]. Whether the β -diketones present in berry wax hold taxonomic significance remains to be determined in the different fruit types that contain them.

The esters in fruit wax are mainly alkyl esters, ketoesters, aromatic esters, estolides (fatty acid polyesters), and glycerides. The presence of some fatty acid methyl esters has been reported in the cuticular wax of table grapes [41]. Other alkyl esters detected in fruit wax include ethyl palmitate, ethyl linoleate, ethyl oleate, ethyl linolenate, and hexyl palmitate, which are present in apple peel [37]. Estolides are a class of polyesters formed by an esterification reaction between two or more hydroxy fatty acid molecules. Glycerides are typically present in the form of monoglycerides, diglycerides, or triglycerides, and have been found in the wax of blueberry [32].

Most aldehydes are primary aldehydes with an even number of carbon atoms. The wax of bilberry contains 10% aldehydes, represented mostly by octacosanal as the dominant aldehyde, followed by hexacosanal and triacontanal [55]. The aldehyde content has been found to be the highest in the cuticular wax of citrus peels [28], with much lower levels being found in apple [38] and lingonberry [26] waxes, suggesting that aldehydes differ in different types of fruits.

2.2.2. Triterpenoids

Basic triterpenoid skeleton structures have been reported in more than 200 species, although relatively few are present in plant cuticular wax. Triterpenoids can be classified according to their

Table 1
The main epicuticular wax morphological types in fruit peel.


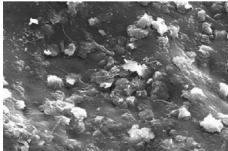
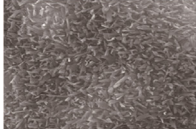
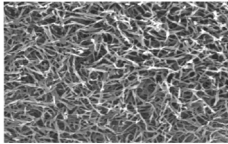
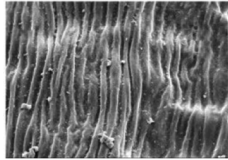
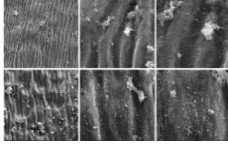
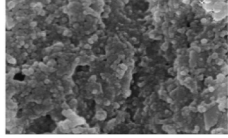
Morphological types	Species	Family	References	
<p>Amorphous films</p>  <p>Reproduced from Ref. [19]</p>	<i>Lycopersicon esculentum</i>	Solanaceae	[20]	
	<i>Mangifera indica</i>	Anacardiaceae	[21]	
	<i>Olea europaea</i> cv. Carboncella	Oleaceae	[17]	
	<i>Vaccinium corymbosum</i> Bluecrop	Ericaceae	[22]	
	<i>Pyrus sinkiangensis</i> Yü. Kuerle	Rosaceae	[23]	
	<i>Pyrus bretschneideri</i> Rehd. Xuehua	Rosaceae	[23]	
	<i>Pyrus bretschneideri</i> Kuerle × Xuehua	Rosaceae	[23]	
	<i>Malus domestica</i> Borkh. Florina and Prima	Rosaceae	[19]	
	<i>Prunus avium</i> cv. Hongdeng	Rosaceae	[24]	
	<i>Olea europaea</i> cv. Ascolana tenera	Oleaceae	[17]	
	<p>Plates/platelets</p>  <p>Reproduced from Ref. [25]</p>	<i>Vaccinium vitis-idaea</i>	Ericaceae	[26]
		<i>Empetrum nigrum</i>	Empetraceae	[26]
		<i>Malus domestica</i> Borkh.	Rosaceae	[27]
		<i>Lycium barbarum</i> (goji)	Solanaceae	[15]
<i>Citrus sinensis</i>		Rutaceae	[25,28]	
<i>Citrus unshiu</i>		Rutaceae	[28,29]	
<i>Fortunella crassifolia</i> Swingle cv. Suichuan		Rutaceae	[30]	
<i>Olea europaea</i> cv. Cucco, Gentile di Chieti, Dritta, Kalamata, Castiglione, Intosso, and Cassanese		Oleaceae	[17]	
<i>Pyrus sinkiangensis</i> Yü. Kuerle		Rosaceae	[23]	
<i>Prunus laurocerasus</i>		Rosaceae	[31]	
<i>Vaccinium myrtillus</i>		Ericaceae	[26]	
<i>Vaccinium vitis-idaea</i>		Ericaceae	[26]	
<i>Lycium barbarum</i> (goji) Z44 and Z168		Solanaceae	[15]	
<p>Rods/rodlets</p>  <p>Reproduced from Ref. [22]</p>		<i>Olea europaea</i> cv. Cassanese	Oleaceae	[17]
	<i>Vaccinium corymbosum</i> Bluecrop	Ericaceae	[22]	
	<i>Pyrus bretschneideri</i> Rehd. Xuehua	Rosaceae	[23]	
	<i>V. uliginosum</i>	Ericaceae	[26]	
	<i>Vaccinium myrtillus</i>	Ericaceae	[26]	
	<i>Lycium barbarum</i> (goji) Ningnongqi-9	Solanaceae	[15]	
	<p>Tubes</p>  <p>Reproduced from Ref. [18]</p>	<i>Vaccinium corymbosum</i> cv. Misty, O'Neal and Sharpblue	Ericaceae	[18]
<i>Vaccinium corymbosum</i> cv. Brigitta, Darrow and Legacy		Ericaceae	[18]	
<i>Vaccinium ashei</i> cv. Britewell, Premier and Powderblue		Ericaceae	[18,32]	
<p>Lamellae</p>  <p>Reproduced from Ref. [15]</p>	<i>Lycium barbarum</i> (goji)	Solanaceae	[15]	
	<p>Reticulum</p>  <p>Reproduced from Ref. [15]</p>	<i>Lycium barbarum</i> (goji) Ningqi-1 and Ningqi-5	Solanaceae	[15]
<p>Granules/ovate crystals</p>  <p>Reproduced from Ref. [16]</p>		<i>Lycium barbarum</i> (goji) Ningnongqi-9 and 16–23–7–8	Solanaceae	[15]
	<i>Pyrus sinkiangensis</i>	Rosaceae	[16]	
	Qiubai, Kuerle, Clapp Favorite, and Jinfeng			

Table 2
Cuticular wax composition and content in common fruit.

Species	Wax amount ($\mu\text{g}\cdot\text{cm}^{-2}$)	Wax composition	References
Apple	366.00–2185.00	Alkanes, alcohols, fatty acids, terpenes, esters, and aldehydes	[27,35–38]
Bilberry	108.50	triterpenoids, alkanes, fatty acids, aldehydes, primary alcohols, and ketones	[26]
Bog Bilberry	331.30	Fatty acids, ketones, aldehydes, triterpenoids, primary alcohols, and alkanes	[26]
Lingonberry	871.10	Triterpenoids, fatty acids, alkanes, primary alcohols, and aldehydes	[26]
Crowberry	921.80	Alkanes, fatty acids, triterpenoids, aldehydes, primary alcohols, and ketones	[26]
Cranberry	340.00	Triterpenoids, aldehydes, alkanes, fatty acids, alcohols, and sterols	[39]
Blueberry	48.00–332.00	Triterpenoids, β -diketones, aldehydes, primary alcohols, fatty acids, and alkanes	[18,40]
Citrus	3.80–8.20	Triterpenoids, aldehydes, fatty acids, alkanes, and alcohols	[2,28,29]
Grape	61.60–71.60	Fatty acids, alkanes, phenols, alcohols, ketones, and aldehydes	[4,41]
Guava	37.38	Fatty acids, triterpenoids, primary alcohols, alkanes, aldehydes, secondary alcohols, and ketones	[42]
Jujube	172.00–368.00	Fatty acids, primary alcohols, alkanes, triterpenoids, Amines, aldehydes, phenols, esters, and ketones	[43]
Lemon	1.36	Alkanes, aldehydes, alcohols, and fatty acids	[44]
Olive	No data	Esters, alkanes, alcohols, aldehydes, fatty acids, and triterpenoids	[45]
Peach	518.00	Triterpenoids, alkanes, fatty acids, alcohols, and sterols	[46]
Persimmon	337.00–770.00	Triterpenoids, alkanes, and alcohols	[47,48]
Pear	653.00–1431.00	Alkanes, primary alcohols, aldehydes, fatty acids, terpenoids, and esters	[23,49]
Sweet cherry	20.09–59.77	Triterpenoids, alkanes, fatty acids, sterols, and alcohols	[34,50]
Tomato	14.60–17.90	Alkanes, triterpenoids, sterols, fatty acids, and alcohols	[51]

carbon chain structure, number and properties of carbon atoms, position of functional groups, and types of triterpenoid derivatives. Most of the triterpenoid compounds found in cuticular wax are oleanane, ursane, and lupane derivatives, with oleanane and ursane being the most abundant in fruit wax (Fig. 1). For example, oleanane and ursane structures have been identified as the dominant triterpenoids in the waxes of blueberry fruits (*Vaccinium corymbosum* and *Vaccinium ashei*) [18] and tomato fruits (*Solanum lycopersicum* L.) [51]. Oleanane structures are the predominant triterpenoids in grapes [41] and olives [45], while ursolic acid, oleanolic acid, and α/β -amyrin are typically the major triterpenoids in apples [56] and sweet oranges (*Citrus sinensis*) [2]. Small amounts of other triterpenoid derivatives, such as alkyl ethers, alkyl esters, and acyl esters, have also been detected in fruit wax;

for example, lupeol acetate and 3-(acetyloxy)-(3 β)-urs-12-en-28-al were detected in the cuticular wax of Asian pears [23].

2.2.3. Other fruit wax components

In addition to the main components present in the cuticular wax of fruits, as described above, other substances categorized as sterols and flavonoids have been reported. Sterols, including β -sitosterol and stigmasterol, have been identified in the cuticular wax of cranberries, tomatoes, and sweet cherries. These structures are very similar to pentacyclic triterpenes, and they can be proactively or passively transported to different parts of the plant cuticular layer. Plant sterols can accumulate on the cytoplasmic membrane, which allows the sterol in plant tissue to become concentrated [57]. In special cases, other chemical components may

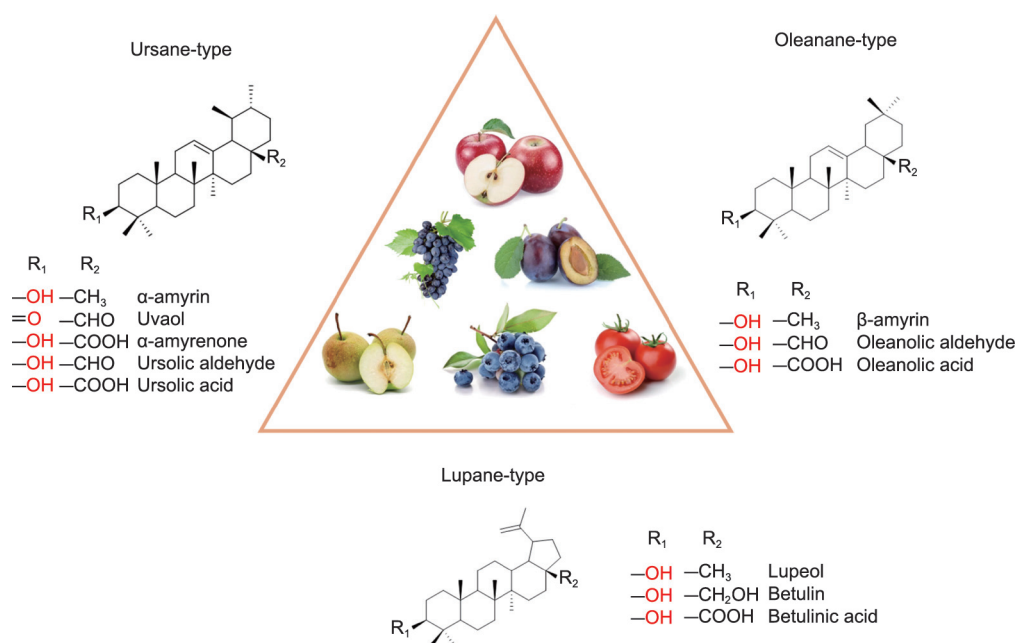


Fig. 1. The main triterpenoids identified in the cuticular wax of fruit. The triterpenoids shown here can be classified based on their structure into triterpenoid acids, alcohols, aldehydes, and ketones that are structurally derived from oleanane, ursane, and lupane.

also appear on a plant surface outside of the wax. Such substances include diterpenoids and natural tocopherol (α -, γ -, and ϵ -tocopherol) [49].

3. The metabolic pathway and its regulation of cuticular wax

3.1. The metabolic pathway of cuticular wax

Based on the composition of a plant's cuticular wax, the biosynthesis pathway of the plant wax is mainly divided into two aspects: the biosynthetic pathway of VLCFAs and their derivatives; and the biosynthetic pathway of triterpenoid cyclic compounds. The former is the normal biosynthetic pathway that forms long-chain aliphatic compounds such as fatty acids, alcohols, esters, alkanes, aldehydes, and ketones. The pathway to form the triterpenoid cyclic compounds in fruit wax can form different types of triterpenoids and their derivatives, such as triterpene alcohols, triterpene aldehydes, triterpene ketones, and triterpene acids. The detailed pathway of cuticular wax biosynthesis and transport is shown in Fig. 2. These wax components are biosynthesized in the cytoplasm of epidermal cells.

The biosynthetic pathway of VLCFAs and their derivatives has been largely elucidated in model plants such as *Arabidopsis* and tomato. First, C16 and C18 acyl carrier proteins (C16/C18 acyl-ACP) are biosynthesized through *de novo* fatty acid synthesis under the catalysis of fatty acid synthase complex (FAS) in the plastid. Next, C16/C18 acyl-ACP is converted into free C16 and C18 fatty acids by acyl-ACP thioesterase (FAT). The C16 and C18 fatty acids, which are the main precursors of VLCFA and derivative synthesis, are exported to the endoplasmic reticulum and esterified with long-chain acyl coenzyme A synthetase (LACS) to form C16 and C18 acyl-CoA. Subsequently, VLCFAs (mainly C20–C34) are

synthesized by the fatty acid elongase complex (FAE). Then, VLCFA derivatives are formed via the acyl-reduction pathway and decarbonylation pathway. Among these derivatives, primary alcohols and esters are formed via the acyl-reduction pathway, while alkanes, secondary alcohols, and ketones are formed via the decarbonylation pathway. Triterpenoids in plants are mainly derived from isopentenyl pyrophosphate (IPP, C5), which is generated via the mevalonate (MVA) pathway that proceeds with acetyl-CoA as the starting substrate in the cytoplasm [58]. IPP is converted to squalene (C30) under the action of geranyl pyrophosphate synthetase (GPS), farnesyl pyrophosphate synthase (FPS), and squalene synthase (SQS), followed by epoxidation with squalene epoxidase (SQE) to produce 2,3-oxidosqualene (C30). Various triterpenoids can be synthesized from 2,3-oxidized squalene through cyclization, hydroxylation, glycosylation, and other structural modifications. The enzymes that mediate these reactions include oxidosqualene cyclases (OSCs), cytochrome P450 monooxygenases (CYP450s), and glycosyltransferases (UGTs). These wax components are transported through the Golgi network and secreted to the outer epidermis of plants under the combined action of adenosine triphosphate (ATP)-binding cassette transporter (ABC transporter) and lipid transfer protein (LTP) [3,59]. The whole process requires dozens of enzymes at least, and hundreds of genes are involved in such regulation [33].

3.2. Structure genes involved in cuticular wax metabolism

Several key genes are involved in fruit wax metabolism. Common structural genes and gene functions reported in fruits are displayed in Table 3 [23,28,46,60–77]. The structural genes from the eceriferum (CER) family have been studied most widely. Albert et al. [60] successfully cloned four structural genes (*CER1*, *CER4*,

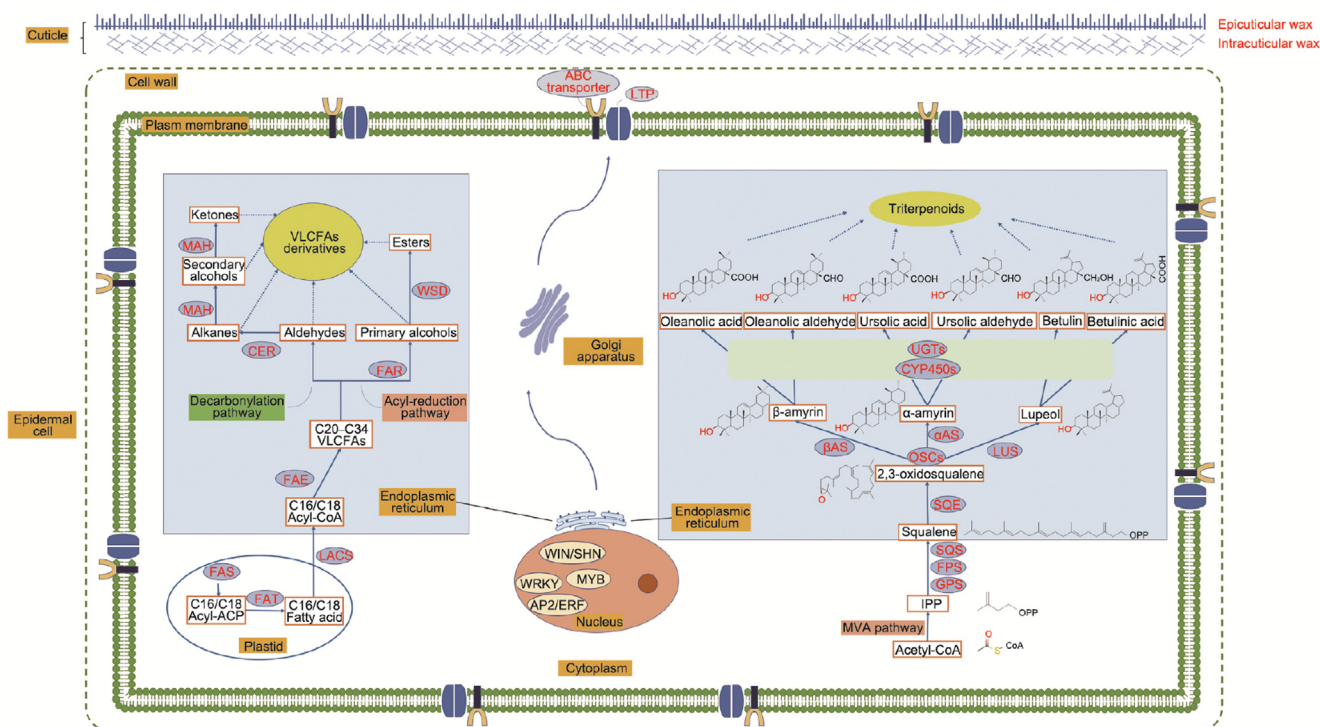


Fig. 2. Synthesis and transport of fruit cuticular wax. LTP: lipid transfer protein; FAS: fatty acid synthase complex; FAT: acyl-ACP thioesterase; LACS: long-chain acyl coenzyme A (CoA) synthetase; FAE: fatty acid elongase complex; ABC transporter: adenosine triphosphate (ATP)-binding cassette transporter; FAR: fatty acyl-CoA reductase; WSD: wax ester synthase/diacylglycerol acyltransferase; CER: fatty-aldehyde decarbonylase; MAH: mid-chain alkane hydroxylase; MVA pathway: mevalonic acid pathway; IPP: isopentenyl diphosphate; GPS: geranyl pyrophosphate synthase; FPS: farnesyl pyrophosphate synthase; SQS: squalene synthase; SQE: squalene epoxidase; OSCs: oxidosqualene cyclases; LUS: lupeol synthase; α AS: α -amyrin synthase; β AS: β -amyrin synthase; CYP450s: cytochrome P450 monooxygenases; UGTs: glycosyltransferases. WIN/SHN, WRKY, MYB, AP2/ERF are transcription factors.

Table 3
Structure genes and transcription factors (TFs) related to wax synthesis and transport regulation in fruit peel.

Gene name	Species	Gene function	References
<i>LeSITTS1</i>	Tomato	β -amyirin synthesis	[65]
<i>LeSITTS2</i>	Tomato	Oxidosqualene cyclase	[65]
<i>PaCER1</i>	Sweet cherry	Formation of very long-chain (VLC) alkanes	[66]
<i>CsCER1</i>	Cucumber	—	[61]
<i>CsCER3</i>	Citrus	—	[64]
<i>CER4</i>	Citrus	Formation of VLC primary alcohols	[60,67]
<i>CsCER4-like1/CsCER4-like3</i>	Citrus	—	[46]
<i>CsCER4</i>	Cucumber	Formation of VLC primary alcohols	[62]
<i>LeCER6</i>	Tomato	β -ketoacyl-CoA synthase VLCFA elongation	[63]
<i>CsCER6</i>	Citrus	β -ketoacyl-CoA synthase Affects the smoothness of citrus fruit epidermis	[28,64]
<i>MdCER6</i>	Apple	β -ketoacyl-CoA synthase VLCFA elongation	[67]
<i>CS-FAD</i>	Cucumber	ω -3-fatty acid desaturase Regulating the conversion of linoleic acid to linoleic acid	[68]
<i>PaKCR1</i>	Sweet cherry	β -ketoacyl-CoA reductase VLCFA elongation	[66]
<i>MdKCS</i>	Apple	β -ketoacyl-CoA synthetase VLCFA elongation	[69]
<i>CsKCS19/CsKCS20/kcs11-like1</i>	Citrus	Biosynthesis of VLC acyl-CoA	[64]
<i>KCS9/KCS20</i>	Pear	β -ketoacyl-CoA synthetase	[23]
<i>MAH1</i>	Pear	Mid-chain alkane hydroxylase, accumulation of secondary alcohols, and ketones	[23]
<i>MdMAH1</i>	Apple	—	[67]
<i>PaLACS2</i>	Sweet cherry	Long-chain acyl-CoA synthetase Biosynthesis of C16 or C18 acyl-CoA	[66]
<i>LACS2</i>	Apple	—	[60]
<i>LACS2</i>	Pear	—	[23]
<i>LACS4</i>	Citrus	Long-chain acyl-CoA Synthetase	[64]
<i>CsCER7</i>	Cucumber	3'-5' exoribonuclease Accumulation of fruit cuticular wax	[70]
<i>CER2</i>	Pear	Participation in carbon atom distribution	[23]
<i>MdWSD1</i>	Apple	Wax ester synthase (WS) and diacylglycerol acyltransferase	[67]
<i>CsABCG11</i>	Citrus	Secretion and transport of aliphatic wax components	[64]
<i>LTPG1</i>	Apple	Secretion and transportation of wax components	[60]
<i>LTPG1</i>	Pear	—	[23]
<i>LTP4</i>	Pear	Encoding lipid transfer protein	[23]
<i>MdMYB30</i> (MYB)	Apple	TFs related to wax accumulation	[71]
<i>MYB96</i> (MYB)	Citrus	—	[28]
<i>MYB16/96/106</i> (MYB)	Pear	—	[23,72]
<i>WRKY20/89</i> (WRKY)	Pear	—	[72]
<i>WIN1</i> (WIN1/SHN1)	Apple	—	[60]
<i>MdSHN3</i> (WIN1/SHN1)	Apple	—	[73]
<i>SISHN1</i> (WIN1/SHN1)	Tomato	—	[74]
<i>CpSHN1/CpSHN2</i> (WIN1/SHN1)	Papaya	—	[75]
<i>WRI4</i> (AP2/SHEN)	Pear	—	[72]
<i>McWRI1</i> (AP2/SHEN)	Apple	—	[76]
<i>MdERF2</i> (AP2/ERF)	Apple	—	[77]

KCS7/2, and *LACS2*) that were specially expressed in apple peel. The *KCS7/2* and *LACS2* genes play an important role in the synthesis of VLCFAs, while the *CER1* and *CER4* genes regulate the synthesis of alkanes and primary alcohols, respectively. Overexpression of the *CsCER1* gene promotes alkane biosynthesis and drought tolerance in cucumber [61]; the *CsCER4* gene is related to the biosynthesis of fatty alcohol [62], and similar conclusions have been verified in apple [60] and citrus [46]. The content of VLCFAs above C28 in the cuticular wax of the tomato *LeCER6*-defective mutant was found to be significantly lower than that of wild-type tomatoes [63]. The *CsCER6* gene is also involved in the wax biosynthesis

pathway and affects the smoothness of citrus fruit peel [64]. The transport and secretion of cuticular wax components requires the participation of ABC transporter and LTP. A decrease in *CsABCG11* gene expression in citrus can affect the content of the aliphatic compounds [64].

3.3. Regulation of the cuticular wax metabolism

In recent years, some evidence has shown that the synthesis and transport of plant wax can be regulated at the transcription level [10,78]. Many transcription factors (TFs), such as MYB, WRKY,

and AP2/SHEN, participate in the regulation of plant wax synthesis and transport (Table 3). Zhang et al. [71] identified a new MYB family member, *MdMYB30*, from apple. *MdMYB30* is bound to the *MdKCS1* gene promoter to activate *MdKCS1* gene transcription expression, resulting in the accumulation of cuticular wax. *MYB96* shows a higher expression level in Newhall oranges than in Satsuma oranges, which implies that *MYB96* plays a crucial role in the regulation of wax deposition in citrus fruits [28]. Other MYB family members, such as *MYB16/96/106*, are related to the regulation of cuticular wax biosynthesis in pear cultivars [23,72]. Even though the expression levels of *WRKY20*, *WRKY89*, and *WRI4* (AP2/SHEN family) were not found to be consistent with changes in the wax of three pear cultivars, these genes may regulate wax accumulation through other levels, such as post-transcriptional and post-translational levels [72]. In apples, *McWRI1* activates the promoter of the *McKCS*, *McLAC*, and *McWAX* genes to upregulate their expression levels, resulting in an accumulation of alkanes and a change in the wax structure on the fruit surface [76]. Overexpression of *MdERF2* was found to upregulate the expression levels of *MdLACS2*, *MdWSD1*, *MdCER4*, and *MdCER6* between the plant calli and the fruit to increase the total wax, alkane, and alcohol production, but decreased the acid and ester contents and altered the microstructure of the fruit's cuticular wax [77]. The *MdSHN3* gene, which encodes the SHN1/WIN1 TFs, also positively regulated cuticular wax deposition in apple [73].

In addition to transcription regulation, regulation at the post-transcriptional and post-translational levels plays an important role in plant wax biosynthesis and transport. However, few studies have focused on these post-transcriptional regulatory mechanisms [78]. Some results in *Arabidopsis* indicate that cuticular wax biosynthesis is regulated by RNA exosome and RNA-mediated gene silencing machinery [79,80]. Moreover, the components of the SUPERKILLER (Ski) complex, cytosolic exosome cofactors, and microRNAs are also involved in the regulation of cuticular wax biosynthesis in *Arabidopsis*, rice (*Oryza sativa*), and wheat (*Triticum turgidum* ssp. *durum*) [81,82]. As for the post-translational modification mechanism of cuticular wax biosynthesis, there is some evidence on protein ubiquitination and the 26S proteasome system [83,84]. However, there is no reported research on the post-transcriptional and post-translational regulation of cuticular wax in fruit quality. Thus, these fields require further study.

4. Effects of storage conditions on cuticular fruit wax

The synthesis and transport of cuticular wax not only occur at the fruit development and ripening stage but are also carried out after harvest. Many postharvest conditions, such as temperature, relative humidity (RH), gas atmosphere, and exogenous hormones, regulate cuticular wax metabolism in postharvest fruits.

4.1. Temperature

Studies have shown that the temperature can affect cuticular wax morphology and composition in fruits during storage [2]. The total wax in apple fruits was found to be richer at room-temperature storage (25 °C) than at low-temperature (4 °C) storage. During storage at 4 °C, the epicuticular wax crystals changed from flattened platelets to small granule-like wax particles and then to a melting morphology; in contrast, after storage at 25 °C, the wax crystals changed from flattened platelets to small granule-like wax particles [2]. It was speculated that the greater accumulation of cuticular wax during room-temperature storage might be due to the higher temperature inducing fatty acid biosynthesis and the decarbonylation pathway. The decrease in cuticle wax during extended long-term cold storage appeared to be

mediated via the decreased expression of genes belonging to the fatty acid elongation complex and the ECERIFERUM proteins 1 and 3 (CER1 and CER3) [85]. In addition, the expression of the *PpCER1*, *PpLACS1*, and *PpLipase* genes was found to be inhibited in peaches during cold storage compared with fruits stored at 20 °C [46].

4.2. Relative humidity

Previous studies have revealed that the fruit cuticle—and, more specifically, the composition of the epicuticular wax layer—is associated with postharvest fruit quality maintenance [5]. After focusing on the RH conditions that caused fruit dehydration after the fruit was taken from the vine, the effects of such stress on wax composition were found in Korla pear [49]. In Korla pears under high humidity storage, the composition of the wax (e.g., alkanes and aldehydes) and the number and morphology of the wax crystals were maintained, which helped preserve water and delay fruit senescence [49]. Nevertheless, RH did not affect the total epicuticular wax load of orange fruits; rather it changed the chemical composition of the wax. Compared with a high RH environment, fruit conservation at a low RH contributes to an increase in the proportion of alcohols and fatty acids in the fruit wax, along with a decrease in terpenoids and alkanes, resulting in a lower alkane/terpenoid ratio, which is related to higher fruit weight loss and cuticle permeability values [53]. However, the tomato was not found to modify its wax load and chemical profile in response to the postharvest dehydrating conditions that led to fruit quality loss [86].

4.3. Controlled gas storage

Carbon dioxide (CO₂) shocks induced changes in cuticle composition and the expression of cuticle-related genes (*PpCER1*, *PpLACS1*, and *PpLipase*) in October Sun peaches [46]. Moreover, an obvious increase was observed in microcracks in the cuticle of apples and changes in the shape of the epicuticular wax under a controlled atmosphere (1.5% O₂, 2.5% CO₂, 0 °C, and 90% RH), as compared with that under a regular atmosphere (0 °C and 90% RH) [87]. Furthermore, Klein et al. [37] reported changes in the wax content and composition of apples during their shelf life after controlled atmosphere (CA) and dynamic controlled atmosphere (DCA) storage. They found that the total wax content increased and the greasiness decreased in apples under DCA treatments, compared with those under a CA. The stored fruits in a DCA exhibited the lowest respiration and highest quality; thus, a DCA was reported to be the best storage condition.

4.4. Ethylene and 1-methylcyclopropene

Variation in fruit wax composition and wax content is predominantly regulated by ethylene phytohormone [88–90]. Navelate oranges exposed to 4 μL·L⁻¹ ethylene for 4 days showed higher expression levels of *CsCER4/CsFAR3* (alcohol formation) and *CsABCG11/WBC11* (wax transport) than the control fruit, while the expression of these genes was down-regulated in fruit treated with 4 μL·L⁻¹ ethylene for 8 days [90]. Ethephon, an ethylene mimic, increased cuticular wax density and accelerated wax crystal melting and fruit senescence during the cold storage of Starkrimson apples [67], whereas the expression of *MdCER6* (VLCFAs synthesis), *MdCER4*, and *MdWSD1* (alcohol formation) were up-regulated. The role of 1-methylcyclopropene (1-MCP), which is an ethylene receptor inhibitor, is often the opposite of that of ethylene in terms of cuticular wax regulation. The wax content of pears treated with 1-MCP was found to be lower than that of the control fruit during the whole storage period, indicating that 1-MCP treatment effectively inhibits the production of wax in the

pear epidermis [91]. 1-MCP was also found to decrease fatty acid and 10-nonacosanol concentrations in Maxi Gala apples [56]. Moreover, the accumulation of liquid constituents in the wax and the greasiness of Cripps Pink apple skin were found to be suppressed in fruit treated with 1-MCP after 70 days of storage at 20 °C [89]. Another negative effect of 1-MCP on wax components was reported by Yang et al. [92], who found that 1-MCP significantly delayed α -farnesene production in Golden Delicious apples during their shelf life at 20 °C.

4.5. Miscellaneous

Other phytohormones, such as β -aminobutyric acid (BABA) [24], methyl jasmonate (MeJA) [72], and abscisic acid (ABA) [53], have been shown to regulate the expression of wax biosynthesis and transport-related genes in postharvest fruits. Wang et al. [24] found that sweet cherries treated with BABA had a smoother cuticle and better integrated subepidermal cell as compared with the control, and that BABA treatment also reduced fruit water loss and delayed the increase of cell membrane permeability and the decrease of fruit firmness. ABA treatment mediated wax compositional changes differently in sweet orange fruits at high or low RH [53]. Moreover, VLCFAs (hexadecanoic acid and hexacosanoic acid) were reported to affect wax coverage and structure in pears, as well as regulating the expression of wax structure genes and TF genes [72]. Postharvest heat treatment was also shown to affect cuticular wax metabolism in peach fruit [46].

5. Function of cuticular wax in postharvest fruit quality maintenance

Cuticular wax is not just a physical barrier that prevents fruits from their environment and from pathogen invasion; it also has a close relationship with fruit quality [93]. The composition, content, and structure of cuticular wax have been found to directly affect the storage quality and disease resistance of fruits, as summarized in Fig. 3 and explained in the next subsections.

5.1. Wax and fruit water loss

Some fruit surfaces in specific fruit types have no pores after ripening. The cuticle is thus considered to be the only barrier controlling water transpiration, by which it regulates the water balance and maintains fruit surface integrity [94]. The wax layer can prevent the non-stomatal loss of water in plant tissues, which is related to the hydrophobicity of the wax and the complex spatial structure. The decarbonylation pathway of wax biosynthesis was found to be blocked in the epidermal cells of the *lecer6* wax mutant of tomato, thereby accelerating water loss in the cuticle of the fruit epidermis [95]. Cuticular wax content, especially alkane content, was found to be an important determinant of water infiltration in postharvest peppers (*Capsicum* spp.) [96]. A correlation analysis of apple wax components and storage quality also found that wax components—especially alkanes—are required to maintain apple fruit quality during the storage period [38]. Alkanes were also found to play an important role in reducing water loss and chilling injury in zucchini fruit during cold storage [97].

Although both intracuticular wax and epicuticular wax affect water dispersion, the intracuticular wax component is the dominant factor [6]. Water loss from the cuticle of dewaxed fruit was more obvious than that in non-waxed fruit, and the water loss of tomatoes with wax removal was significantly higher than that without wax removal [98]. Transpiration water loss in the cuticle of tomatoes largely determined by the aliphatic compounds in intracuticular wax layer, and the aliphatic compounds in epicuticular wax layer played a minor role. Reduction of the intracuticular aliphatics caused more fruit water loss than that removal of the epicuticular wax layer. Aldehydes, fatty acids, alkanes, and primary alcohols in the cuticular wax of oranges (*Citrus sinensis*) and mandarins (*Citrus unshiu*) played important roles in the water permeability of the fruits [28]. The weight loss of European plums was found to be mainly caused by the loss of water in the cuticle (95%), rather than by stomatal transpiration. The water-holding capacity of the intact fruit was stronger than that of fruit with the wax removed (via a physical method or a chemical method). The lower the storage temperature, the slower the fruit water loss [7]. Similar results were obtained in other fruits such as peach [99], citrus [28], and blueberry [12].

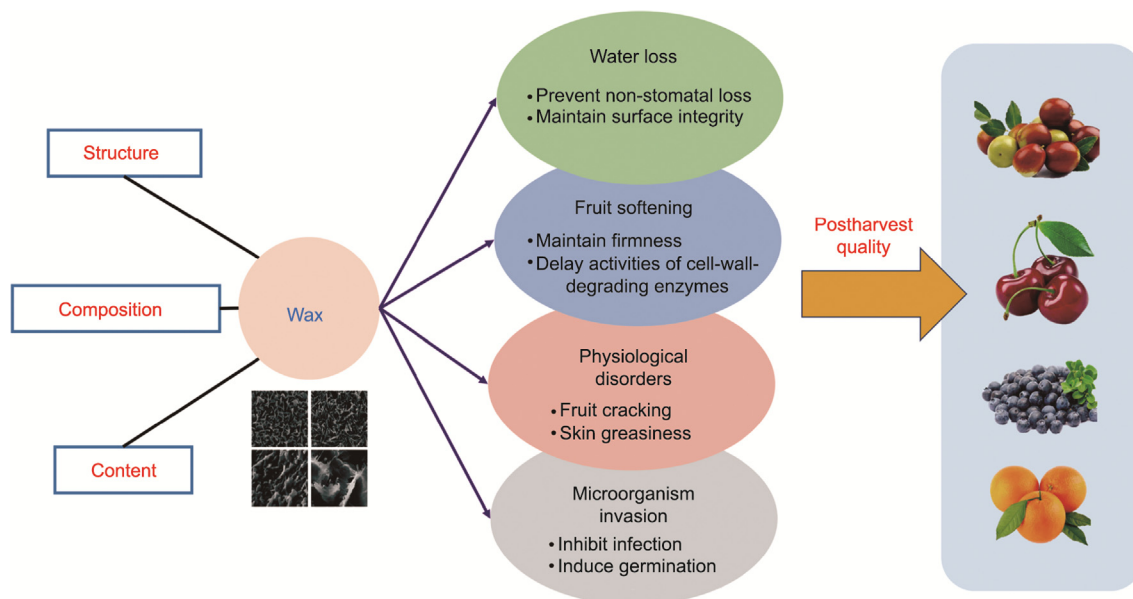


Fig. 3. The role of cuticular wax in fruit quality maintenance. Cuticular wax affects four aspects of fruit maintenance: fruit water loss, fruit softening, fruit physiological disorders, and microorganism invasion.

5.2. Wax and fruit softening

In addition to the cell wall structure, the cuticle layer can maintain fruit firmness to a certain extent, and cuticular wax can also effectively delay the softening process of fruit. The presence of cuticular wax in blueberries was found to significantly reduce the activities of cell-wall-degrading enzymes (i.e., pectinase, polygalacturonase, and cellulase), thereby delaying the degradation of protopectin and cellulose, reducing cell wall degradation, and maintaining fruit firmness; in comparison, the process of softening and senescence intensified in fruit with the wax removed [12]. Some apple varieties that showed little change in firmness and weight loss rate during storage were found to have a thicker and denser cuticular layer, and there was a significant positive correlation between the thickness of the cuticle and the firmness of fruit during storage [100]. Similar results for pear fruit implied that the properties of the cuticle layer—including acid- and alkali-resistance and non-pervasive permeability—effectively maintained the integrity of the epidermal cells, and thereby maintained fruit firmness to a certain extent [101].

5.3. Wax and physiological disorders of fruit

Fruit cracking is a common cause of economic loss in several fruits, such as tomato, litchi, and cherry. Different varieties of cherry have different tolerance to fruit cracking [102]. Fruit cracking is mainly caused by a rainy and high-humidity environment during the fruit maturity stage. Rainwater penetrates into the fruit's cells through the epidermis cuticle. The alkane content in cuticle wax affects the cuticle permeability, where a higher alkane level results in a lower cuticle permeability, thereby reducing the water permeability and giving the fruit greater tolerance to fruit cracking [6]. The occurrence of superficial scald on the surface of apples is also thought to be associated with changes in the cuticular wax. Apple wax contains α -farnesene, among other components, and the accumulation of α -farnesene and its oxidation products may be the cause of superficial scald. High temperature and water shortages increase the accumulation of α -farnesene oxidation products, thus increasing the incidence of superficial scald [103]. The content of fatty acids, esters, and antioxidant substances in peel wax increases under low temperature, and the oxidation of unsaturated fatty acids is inhibited to some extent, thereby increasing cell membrane permeability and reducing the occurrence of superficial scald [103].

In addition, the skin of fruits such as apple and pear becomes greasy during postharvest storage [27,104]. Skin greasiness is a physiological disorder that seriously affects the sensory quality of fruit and may be related to cuticular wax composition and microstructural changes in the pericarp [27]. Christeller and Roughan [105] found that the induction of apple greasiness was correlated with the long-chain unsaturated fatty acid esters of farnesol. Later, Yang et al. [27] revealed that the unpleasant greasy feeling of a stored apple was caused by an accumulation of fluid wax constituents, especially esters of (E,E)-farnesol. In addition, wax alcohols and fatty alcohols may affect the phase change of a wax from a solid to a liquid. The surface of apples stored at room temperature produces new wax, which is positively correlated with the degree of greasiness. It is worth noting that the occurrence of greasy apples does not depend on the production of new wax; rather, changes in some specific wax components affect the greasiness of the fruit skin [106]. Whether the production of new wax is due to wax biosynthesis initiated by the fruit itself or to the transformation of other cuticle components remains to be studied.

5.4. Wax and microorganism invasion

The plant cuticular layer is the first barrier against pathogens invading the plant host. The special structure of the cuticle as a natural barrier plays a physical role in blocking pathogenic microorganisms. In addition, the strongly hydrophobic components of the cuticle can inhibit the production and attachment of pathogenic spores to some extent [107]. Certain antifungal components in cuticular wax, such as triterpenoids, alkanes, and sterols, can also inhibit mycelial growth via their antimicrobial action [108]. Although the total wax content does not differ between susceptible grape varieties and resistant grape varieties, certain specific antifungal components (with an inhibition rate of powdery mildew spores of more than 75%) only exist in resistant grapes and include fatty acids, alkanes, terpenes, indole derivatives, ketones, amines, phenols, and sterols [4].

In contrast, studies have shown that wax components in the plant epidermis can also be specifically recognized by pathogens, and that their hydrophobicity can induce pathogen growth and development (e.g., spore germination, tube elongation, and appressorium differentiation) [109–111]. For example, the cuticle wax of mandarin fruit (*Citrus reticulata* Blanco) inhibits the mycelial growth of *Penicillium digitatum*, but can promote the germination of conidia and elongation of the germ tube [9]. However, fatty acids, alkanes, and terpenes in the epicuticular wax of another mandarin cultivar (*Citrus unshiu*) significantly promote the mycelial growth of *Penicillium* (*in vitro*), although spraying exogenous wax on citrus fruit was found to inhibit mycelial growth (*in vivo*) [29]. Moreover, Tang et al. [111] found that the composition and hydrophobicity of pear cuticular wax contributed to the formation of *Alternaria alternata* infection structure. These two seemingly completely opposite results illustrate the unique role played by wax components in plant infection by pathogens. The reason for these opposite effects may be that different citrus cuticular wax components have different effects on pathogen growth. Identifying how pathogens interact and signal with wax components can help account for this discrepancy. Moreover, the mycelium growth rate of pathogens will differ in different environments (i.e., the fruit surface and the plate medium).

6. Conclusions and future perspectives

Cuticular wax is a special layer of coating on the outside of plant epidermal cells. It has been the focus of plant research in recent decades due to its important role in maintaining fruit quality. Important breakthroughs have been made toward identifying the structure and composition, synthesis and transport regulation, and effects on fruit quality of cuticular wax. Although both wax composition and content are related to the transpiration loss of the cuticle, wax composition plays a major role in comparison with the total amount of wax, as described in this review, and thus should be further examined in the future as a target for fruit quality improvement. Future research on fruit wax should focus on the relationship between wax composition and crystal structure. Questions such as “what kind of wax composition determines the wax morphology?” and “does the wax content affect wax crystal morphology?” remain to be studied.

In addition, the multifunctional roles of cuticular wax in fruit disease resistance need to be further revealed. On the one hand, wax composition inhibits the growth of pathogens, while on the other hand, wax promotes pathogen infection—two seemingly opposite results that may need further exploration in the future. To aid in answering these questions, the production of wax-defective mutant fruit would make it possible to critically assess the role of fruit wax in fruit shelf life and disease resistance.

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Compliance with ethics guidelines

Weijie Wu, Bo Jiang, Ruiling Liu, Yanchao Han, Xiangjun Fang, Mohamed A. Farage, Honglei Mu, Jesus Simal-Gandara, Miguel A. Prieto, Hangjun Chen, Jianbo Xiao, and Haiyan Gao declare that they have no conflict of interest or financial conflicts to disclose.

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