European Journal of Lipid Science and Technology

WILEY-VCH

European Journal of
Lipid Science and Technology

RESEARCH ARTICLE OPEN ACCESS

Major Components of Epicuticular Wax From Fruit of the Canadian Prairies: Saskatoon Berry (*Amelanchier alnifolia*), Canadian Dwarf Sour Cherry (*Prunus* × *kerrasis*), Haskap (*Lonicera caerulea*), and Red Twinberry (*Lonicera utahensis*)

Daniel Hupka[1] | Haixia Zhang[2] | Mark A. Smith[1]

[1]Agriculture and Agri-Food Canada, Saskatoon Research and Development Centre, Saskatoon, Saskatchewan, Canada | [2]Department of Food and Bioproduct Sciences, University of Saskatchewan, Saskatoon, Saskatchewan, Canada

Correspondence: Mark A. Smith (Mark.Smith2@agr.gc.ca)

Received: 27 November 2025 | **Revised:** 24 March 2026 | **Accepted:** 30 April 2026

Keywords: *Amelanchier alnifolia* | cherry | epicuticular wax | haskap | *Lonicera caerulea* | nonacosan-10-ol | ursolic acid

ABSTRACT

Surface wax of fruit plays an important role in post-harvest quality but there are knowledge gaps concerning the wax composition of Canadian summer fruit. Waxes were extracted from ripe, freshly picked fruit of Saskatoon berry (*Amelanchier alnifolia*), Canadian dwarf sour cherry (*Prunus* × *kerrasis*), haskap (*Lonicera caerulea*), and red twinberry (*L. utahensis*) using a chloroform wash. Extracts were separated by GC for quantification by FID and identification by mass spectrometry. The major components of Saskatoon berry wax were nonacosan-10-ol and nonacosane, accounting for 39% and 18% of the wax, respectively. Additional components included free fatty acids, primary alcohols, aldehydes, and triterpenoids including α-amyrin and ursolic acid. Canadian sour cherry wax was enriched in the pentacyclic triterpenoids (60%–63%) ursolic acid, and oleanolic acid, together with nonacosane (20%–23%). Two varieties of haskap were surveyed, differing in primary alcohol content, but sharing nonacosan-10-ol and nonacosan-5,10,-diol as their major components. Analysis of red twinberry as an additional member of the genus *Lonicera*, is the first reported for this species and revealed a wax rich in triterpenoids, illustrating diversity of composition within the genus. The findings of this pilot study will aid in obtaining a better understanding of the role of wax components in aspects such as berry shelf life and consumer appeal. Identifying chemical diversity of wax in berries from plants of the same or related species could be of value in breeding for improved varieties.

Practical applications: Knowledge of surface wax composition will aid in understanding the function of components of the wax layer in controlling water loss and possible roles in biotic interactions such as with insects and with fungal and microbial pathogens. It also provides information on potential health promoting compounds associated with the fruit surface. Identification of wax diversity within haskap suggests strong potential for wax modification through breeding. The work adds significant new information to species where wax composition is either uncharacterized, or not well described. These plants have promise for widespread production in temperate regions.

Abbreviations: BSTFA, N,O-bis[trimethylsilyl]trifluoroacetamide; GC, gas chromatography; GC/FID, gas chromatography with flame ionization detection; GC/MS, gas chromatography with mass spectrometry; TMCS, trimethylchlorosilane; VLCFA, very-long-chain fatty acid.

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2026 His Majesty the King in Right of Canada and The Author(s). *European Journal of Lipid Science and Technology* published by Wiley-VCH GmbH. Reproduced with the permission of the Minister of Agriculture and Agri-Food.

1 | Introduction

In common with other aerial plant surfaces, fleshy fruit are covered by a cuticle, a matrix of cutin and waxes produced by epidermal cells [1]. The cuticle and cuticular wax play an important role in the shelf life and appearance of harvested fruit [2, 3]. Cuticle thickness and structure, together with the embedded waxes are considered to be factors controlling water loss and resulting shriveling and cracking. In fruit with a noticeable wax bloom, surface wax influences appearance, color, and perceived fruit quality. The blue color of highbush blueberries (*Vaccinium corymbosum* L.), for example, is due to the light scattering properties of epicuticular wax crystals overlaying the dark, anthocyanin rich outer skin of the berry [4, 5]. Surface bloom in these fruit is an important attribute influencing consumer preference when offered a selection of varieties [6]. Cuticle thickness and wax composition may also be involved in protecting harvested fruit from microorganism mediated decay [2]. Factors playing a role include the hydrophobic nature of the waxes reducing the availability of water for fungal spore germination, and the presence of specific protective chemicals within the wax. As an illustration, ursolic acid, a triterpenoid often found in berry waxes has recently been shown to inhibit the growth of *Botrytis cinerea*, a common pathogen causing fruit spoilage in storage [7].

Plant surface waxes are diverse in chemical composition but are generally composed of a mixture of very-long-chain fatty acid (VLCFA) derived aliphatic molecules, often together with triterpenoids and minor components such as flavonoids and phenolics [8–10]. Wax levels and composition are strongly influenced by genetic factors, developmental stage, growing environment, and stress [11, 12]. Nonuniform composition of wax over different plant organs has also been reported [13, 14]. The composition of fruit waxes has been studied for many species, often over multiple stages of fruit development, and chemical diversity reflects that seen for other plant organs [1, 15]. Studies also suggest that active synthesis of cutin and wax continues after fruit harvest, and both the amount and composition of these components can be influenced by storage temperature and duration [3, 15–17]. Understanding wax composition and biosynthesis in fruit, with the objective of introducing favorable changes through selective breeding, clearly has considerable potential as an avenue to improving fruit quality and shelf life. In addition, an often overlooked consideration is that the cuticle chemical components can also contribute to the nutritional value of the fruit, and potentially to the value of waste streams from fruit processing, such as pomace from juice extraction.

Due to the relatively harsh climate, there are limited options for fruit production on the Canadian prairies. For this reason there has been a focus on breeding cold hardy varieties of fruit such as sour cherry (*Prunus* × *kerrasis*) and haskap (*Lonicera caerulea* L.), and improving native fruit such as Saskatoon berry (*Amelianchier alnifolia* Nutt.) [18]. Although highly successful, the market for fresh fruit such as haskap is limited by geographic isolation from major population centers and the short shelf life of ripe fruit. Manipulating fruit surfaces, such as altering wax composition to reduce water loss, physical damage, and spoilage, may allow an expanded market for fresh fruit. Unlike more widely grown fruit such as blueberry, the wax composition of prairie fruit is not well characterized. Understanding wax composition, amounts and uncovering potential chemical diversity may provide useful information that can be applied to fruit improvement. As a pilot study towards this objective, ripe fruit were collected from a selection of fruit bearing plants growing on the campus of the University of Saskatchewan to determine the major components of their surface wax. Species chosen were Saskatoon berry, Canadian dwarf sour cherry, and haskap, with red twinberry (*L. utahensis* S. Watson) as a comparative *Lonicera* species.

Saskatoon berry is a deciduous shrub from the Rose family (Rosaceae) native to North America, also known as the Western serviceberry or juneberry [19]. It is grown commercially in Canada, particularly in the province of Saskatchewan. Saskatoon berry fruit turn dark purple when ripe with a slightly glaucous appearance. Fruit can be eaten fresh but are more often processed into products such as jams, pie fillings, and wine. The chemical composition of ripe berries of *A. alnifolia*, and potential health promoting properties, have been studied by other research groups [20–22]. Reports generally focus on the analysis of whole berries and extracts without separate consideration of surface chemicals. Knowledge of the surface wax composition of Saskatoon berry is primarily based on the report of Knowles and coworkers [23]. This work studied the chemical composition of wax, extracted by dipping berries in dichloromethane, at four stages of development. The wax of purple, fully mature Stage 4 fruit was described as comparatively simple, composed of alkanes, primary alcohols, long chain esters, and a secondary alcohol. Two major components, the 29-carbon (C29) alkane nonacosane and the C29 secondary alcohol nonacosan-10-ol comprised almost half of the total wax. Free fatty acids and triterpenoids were not detected and nearly 40% of the wax was categorized as "other" and not clearly identified.

Canadian dwarf sour cherries are cold hardy shrubs bred for cherry production in the Prairie Provinces and are members of the Rose family (Rosaceae) [18]. "Valentine" is a dwarf sour cherry variety from the Romance series, developed by the University of Saskatchewan cherry breeding program led by Dr. Robert H. Bors. The variety produces fruit that are red at ripeness with a glossy appearance and is a hybrid derived through crossing of *P. cerasus* (European sour cherry) and *P. fruiticosa* (Mongolian cherry). Information on the chemical composition of various cherry species is available [24, 25]. As the first dwarf sour cherry varieties, including "Valentine," were only released commercially in 2003, there is relatively little information of the chemical composition of this specific type of cherry. Investigation of surface chemistry, cuticle, and waxes of cherry, has largely focused on sweet cherry (*P. avium*) in relation to shelf life and fruit cracking with the triterpenoid ursolic acid and the alkane nonacosane recognized at the major wax components [1, 15, 26, 27].

L. caerulea also known as blue honeysuckle, honeyberry, or haskap is a perennial fruiting shrub widespread in the northern hemisphere and well adapted to winter survival in cold northern continental climates. Multiple subspecies or varieties are known with diverse morphology and fruit size and shape. Haskap has a long tradition of use as a health promoting fruit in Japan and Russia and improvement and development of the species as a commercial berry crop has been ongoing in a number of locations

including at the University of Saskatchewan, Saskatoon, Canada [28]. The chemical composition of haskap fruit has been studied in detail and haskap is recognized as containing high levels of health promoting compounds such as anthocyanidins, phenolic acids, and ascorbic acid [29–31]. Haskap berries have a noticeable wax bloom on their surfaces that is easily removed during harvest and by post-harvest handling. Only limited information is available concerning the composition of this wax but wax turnover has been suggested to play a role in fruit shelf life during storage [17, 32].

Red twinberry (*L. utahensis* S. Watson), native to Western North America and also known as Rocky Mountain honeysuckle or Utah honeysuckle, is commonly grown as an ornamental shrub for its spring flowers and bright red berries [33]. The berries occur in pairs and are considered edible, although they are rarely eaten due to a bitter aftertaste. For this study, red twinberry was chosen as another representative of the genus *Lonicera*, and because the bush was growing adjacent to one of the Haskap bushes. The chemical composition of red twinberries has not been investigated in detail, although iridoid and secoiridoid glycosides have been reported from closely related *Lonicera* species, *L. morrowii*, and *L. tatarica*, and are thought to act as plant defense compounds [34].

2 | Materials and Methods

2.1 | Fruit Collection and Wax Extraction

Fruit for this study was collected from single plants growing in the grounds of the University of Saskatchewan, Saskatoon campus, and at the AAFC Saskatoon Research and Development Centre. Bushes were all part of formal plantings and were commercial cultivars or releases from the University of Saskatchewan fruit breeding program. With the exception of the sour cherry (Valentine), the varieties are unknown as plants were not labeled and records were unavailable. Haskap was collected on July 3rd 2025. Saskatoon berry, red twinberry, and spruce (young shoots) were collected on July 11th 2025, cherry was collected on July 15th 2025. Samples were collected into brown paper bags using scissors, while avoiding touching the fruit, and were transferred to $CHCl_3$ washed beakers and weighed. To extract wax, two times the volume of $CHCl_3$ was added and the samples were agitated gently for the time given in results. The $CHCl_3$ was then decanted into a fresh beaker and evaporated to dryness under a gentle stream of nitrogen gas. Dried wax was carefully resuspended in $CHCl_3$ and transferred to a 16 mL glass screw top tube. After drying, samples were weighed to determine the weight of collected wax. For analysis, samples in $CHCl_3$ were transferred to glass GC vial inserts, gently dried, and resuspended in 60 µL silanization reagent. The reagent consisted of BSTFA with 1% trimethylchlorosilane (TMCS) in an equal volume of pyridine (Millipore Sigma). After addition of 60 µL hexane:toluene (1:1 v/v), samples were left at room temperature for at least 1 h prior to GC analysis. The BSTFA-TMCS reagent was purchased from Supelco, round Rock, TX, USA, $CHCl_3$ was from Caledon Chemicals, Georgetown, ON, Canada and all other solvents and standards were from MilliporeSigma, Burlington, MA, USA.

2.2 | Separation and Identification by GC/FID and GC/MS

Samples were separated by gas chromatography (GC) using a Trace 1310 gas chromatograph (ThermoFisher Scientific) equipped with two 30 m, 0.25 mm, 0.25 µm DB5-MS capillary columns, a flame ionization detector (FID), and TSQ 9000 Triple Quadrupole Mass Spectrometer (ThermoFisher Scientific). For FID, a 1 µL sample injection volume was used with injection in split mode, a split ratio of 17:1 and injector temperature of 300°C. Hydrogen carrier gas flow was 1.5 mL min^{-1} with constant flow. An initial oven temperature of 125°C was held for 2 min, followed by a ramp of 15°C min^{-1} to 265°C. After a hold of 7 min, a second ramp of 20°C min^{-1} took the temperature to 325°C, with a hold of 38 min which gave a total run time of 59.3 min. Detector temperature was maintained at 300°C. To collect mass spectra, identical inlet and oven conditions were used with the column coupled to the TSQ 9000 triple quadrupole mass spectrometer (MS). The transfer line was maintained at 325°C and ion source at 310°C, operating at an ionization voltage of 70 eV. Data was collected using a mass scan of 40 to 800 *m/z* and a dwell time of 0.2 s. Data were analyzed using Dionex Chromeleon 7 chromatography data system software (version 7.2.9. Thermofisher Scientific) gas chromatograms and mass spectra were copied into Microsoft PowerPoint to prepare the figures. Spectra were edited to alter legend font size for clarity.

3 | Results

3.1 | Saskatoon Berry

Ripe Saskatoon berries, based on dark purple coloring (Figure 1a, arrow), were collected and placed in a glass beaker. Wax was collected by rinsing in $CHCl_3$ for 10 s. The sample of 123.3 g of berries yielded 60.3 mg of wax, giving a wax recovery of 0.49 mg/g of fresh berries. Fruit size and surface area were not measured. A gas chromatography with flame ionization detection (GC/FID) chromatogram showing separation of wax components is presented in Figure 1b,c. Electron impact (EI) mass spectra were collected and identities of 30 of the 36 most abundant peaks were determined. All mass spectra are shown in Supporting Information S1. In agreement with the previous study the C29 alkane nonacosane (17.6%) and the C29 secondary alcohol nonacosan-10-ol (39.3%) were found to be the dominant components of the wax accounting for 56.9% of total wax (Table 1) [23]. For the secondary alcohol, a small $[M-15]^+$ ion at 481.5 *m/z* confirmed the C29 chain length and major fragment ions at 229.3 and 369.4 *m/z*, corresponding to α-cleavage either side of the functional group placed the hydroxyl at C10 (Figure 2a). To further confirm its identity, a $CHCl_3$ soluble wax fraction was prepared from young needles of Colorado blue spruce (*Picea pungens*) where nonacosan-10-ol has been reported as a major component [35]. The mass spectrum from spruce (Figure 2b), displayed the characteristic fragmentation pattern of nonacosan-10-ol, matching that from the Saskatoon berry sample. In addition to nonacosane, other odd-chain, C23, C25, C27, and C31 and even-chain C28 and C30 alkanes were detected at low abundance with total alkanes comprising 19.2% of the wax. Two aldehydes were observed, octacosanal (1.3%) and tricontanal (4%) together

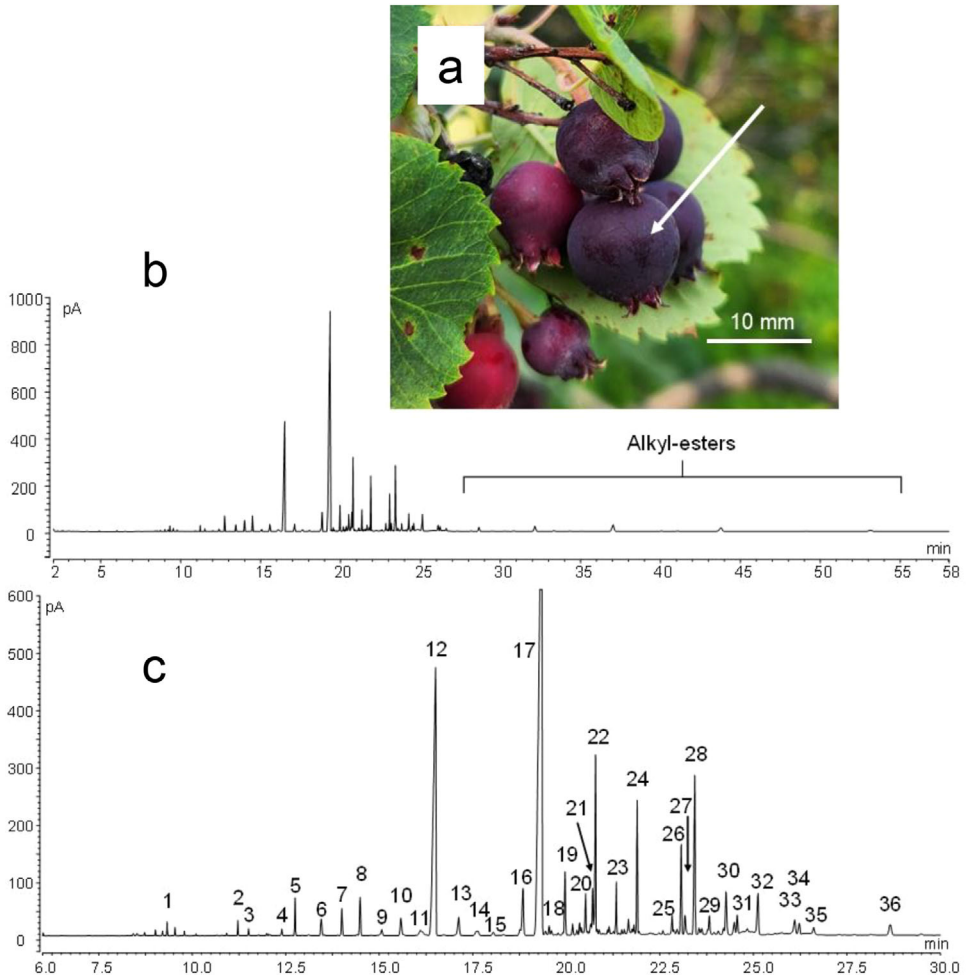


FIGURE 1 | (a) Saskatoon berries indicating ripe berry with an arrow, (b) full chromatogram of Saskatoon berry wax extract, (c) enlarged chromatogram of 6–30 min showing major peaks. Identity of labeled peaks: (1) hexadecanoic acid, (2) tricosane, (3) eicosan-1-ol, (4) pentacosane, (5) docosan-1-ol, (6) docosanoic acid, (7) heptacosane, (8) tetracosan-1-ol, (9) octacosane, (10) tetracosanoic acid, (11) heptacosan-9-ol and heptacosan-8-ol, (12) nonacosane, (13) hexacosan-1-ol, (14) octacosan-10-ol and octacosan-9-ol, (15) triacontane, (16) octacosanal, (17) nonacosan-10-ol, (18) hentriacontane, (19) octacosan-1-ol, (20) nonacosan-7,10-diol, (21) octacosanoic acid, (22) tricontanal, (23) α-amyrin, (24) tricontanoic acid, (25) triterpene (26) oleanolic acid, (27) betulinic acid, (28) ursolic acid, (29) triterpene (30) triterpene (31) C36 alkyl ester, (32) unidentified, (33) unidentified, (34) unidentified, (35) C38 alkyl ester, (36) C40 alkyl ester.

with a series of even-chain primary alcohols ranging in chain length from C20 to C30. As observed previously, octacosan-1-ol was the most abundant primary alcohol at 1.6% of total wax. Free fatty acids comprised 5.2% of the wax with tricontanoic acid (30:0) being the most abundant (2.7%). Peaks identified as heptacosan-8-ol and heptacosan-9-ol isomers (Figure 1c, Peak 11, total 0.4%) and octacosan-9-ol and octacosan-10-ol isomers (Figure 1c, Peak 14, 0.3%) were observed at low abundance (Supporting Information S1). A single alkane-diol, nonacosan-7,10-diol was observed, accounting for 0.9% of the wax. In contrast to the previous study, triterpenoids were detected, accounting for 15.4% of the collected wax. Major components were oleanolic acid ((3β)-3-Hydroxy-olean-12-en-28-oic acid, 2.4%), and ursolic acid ((3β)-3-Hydroxy-urs-12-en-28-oic acid, 5.0%) accompanied by lower levels of α-amyrin ((3β)-Urs-12-en-3-ol, 1.1%), betulinic acid ((3β)-3-Hydroxy-lup-20(29)-en-28-oic acid, 0.6%), and unidentified triterpenes (6.4%). The identity of oleanolic and ursolic acids was confirmed by comparison of GC-MS spectra to those of commercial standards (Figure 2) and published spectra [36–39]. The ester components of the wax were identified as alkyl esters (wax esters), esters of fatty acids, and fatty alcohols. These were not characterized in detail as little information could be determined from the mass spectra due to their low abundance and strong fragmentation. The presence of fragment ions at 257 *m/z* suggested that 16:0 fatty acid ($[FA+1]^+$ = 257 *m/z*) was a major component of the alkyl-esters. Components that could not be identified comprised 2.8% of the total wax.

3.2 | Canadian Dwarf Sour Cherry

For analysis of the surface wax of Canadian dwarf sour cherry "Valentine" three samples (A, B, and C), each of 30 ripe but firm cherries (Figure 3a), were collected from separate trees. Average cherry weight was calculated to be 2.67 ± 0.4 g with an average diameter of 17.0 ± 0.7 mm. Assuming the cherries to be spherical in shape, the average surface area for a cherry was calculated to be approximately 900 mm^2 ($4\pi r^2$, where r = 8.5 mm). Each sample

TABLE 1 | Composition of wax extracts from Saskatoon berry, Canadian dwarf sour cherry, haskap, red twinberry, and young shoots of Colorado blue spruce (*Picea pungens*).

		Percent of total wax										
Name	**Chain length**	**Saskatoon berry**	**Cherry Sample A**	**Cherry Sample B**	**Cherry Sample B extraction 2**	**Cherry Sample C, hexane fraction**	**Cherry Sample C, $CHCl_3$ fraction**	**Cherry Sample C second extraction**	**Haskap 1**	**Haskap 2**	**Red twin berry**	***Picea pungens***
Tricosane	C23	0.2										
Pentacosane	C25	0.1										
Heptacosane	C27	0.7	0.8	0.7	0.4	1.9	0.0	0.4	0.1	TR	0.6	
Octacosane	C28	0.2	0.6	0.9	0.5	2.3	0.0	0.6				
Nonacosane	C29	17.6	21.4	23.1	11.3	64.4	2.1	13.8	9.7	5.8	10.4	
Triacontane	C30	0.2	1.2	1.8	0.9	5.2	0.0	1.1	0.1	0.2		
Hentriacontane	C31	0.2	2.7	3.7	1.7	10.8	0.0	2.2	3.1	3.1	0.5	
Total alkanes		**19.2**	**26.7**	**30.3**	**14.7**	**84.6**	**2.1**	**18.2**	**13.0**	**9.1**	**11.5**	
Octacosanal	C28	1.3										
Tricontanal	C30	4.0										
Total aldehydes		**5.3**										
Hexadecanoic acid	C16	0.2	0.1	0.4	0.5	0.4	0.1	0.6	TR	0.1		
Octadecanoic acid	C18		0.2	0.4	0.5	0.4	0.2	0.5	TR	0.1		
Docosanoic acid	C22	0.6									0.3	
Tetracosanoic acid	C24	0.7							0.1	0.1	1.3	0.6
Hexacosanoic acid	C26								0.1	TR		
Octacosanoic acid	C28	1.0										
Tricontanoic acid	C30	2.7							0.7	0.9		1.3
Total Free Fatty Acids		**5.2**	**0.3**	**0.9**	**1.1**	**0.9**	**0.3**	**1.1**	**0.9**	**1.2**	**1.6**	
Eicosan-1-ol	C20	0.1										
Docosan-1-ol	C22	0.7							0.2	TR		
Tetracosan-1-ol	C24	1.1							1.6	0.1	2.7	
Hexacosan-1-ol	C26	0.9							2.1	0.2	3	
Octacosan-1-ol	C28	1.6							2.7	0.4	2.2	
Triacontan-1-ol	C30								1.6	1.2		

(Continues)

TABLE 1 | (Continued)

Name	Chain length	Percent of total wax										
		Saskatoon berry	Cherry Sample A	Cherry Sample B	Cherry Sample B extraction 2	Cherry Sample C, hexane fraction	Cherry Sample C, $CHCl_3$ fraction	Cherry Sample C second extraction	Haskap 1	Haskap 2	Red twin berry	*Picea pungens*
Total Primary alcohols		**4.4**							**8.2**	**1.9**	**7.9**	
Heptacosan-8-ol and Heptacoan-9-ol	C27	0.4										
Heptacosan-9-ol	C27								0.1	TR	0.6	
Heptacosan-10-ol	C27											0.3
Octacosan-9-ol and octacosan-10-ol	C28	0.3										
Octacosan-10-ol	C28								0.1	0.2		
Nonacosan-10-ol	C29	39.3	0.1	2.8	1.0	5.0	1.3	1.0	52.3	56.5	24.4	53.0
Nonacosen-10-ol	C29								0.4	0.1		
Triacontan-10-ol									0.2	0.6		
Hentriacontan-11-ol and hentriacontan-12-ol	C31		0.5	0.7	0.6	0.6	0.7	0.6				
Total secondary alcohols		**40.0**	**0.6**	**3.5**	**1.6**	**5.6**	**2.1**	**1.6**	**53.1**	**57.4**	**25**	
Nonacosan-4,9-diol	C29								0.8	1.5		
Nonacosan-4,10-diol	C29											1.9
Nonacosan-5,10-diol	C29								11.4	17.2	0.8	9.0
Nonacosan-7,10-diol	C29	0.9							1.9	2	0.8	2.4
Nonacosan-8, 11- diol	C29								0.6	0.8	0.6	
Nonacosan-10,13-diol	C29								0.7	0.8		5.1
Total secondary alkane diols		**0.9**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**0.0**	**15.4**	**22.3**	**2.2**	**18.4**
Abietic acid												0.7

(Continues)

TABLE 1 | (Continued)

Name	Chain length	Percent of total wax										
		Saskatoon berry	Cherry Sample A	Cherry Sample B	Cherry Sample B extraction 2	Cherry Sample C, hexane fraction	Cherry Sample C, $CHCl_3$ fraction	Cherry Sample C second extraction	Haskap 1	Haskap 2	Red twin berry	*Picea pungens*
Total diterpenes												**0.7**
α-Amyrin		1.1							0.1	0.2		
oleanolic acid		2.4	11.1	13.3	16.2	1.0	18.4	15.3			6.4	
betulinic acid		0.6									3.6	
Ursolic acid		5.0	46.5	42.2	48.8	2.7	65.6	46.9			31.5	
Maslinic acid			1.6	1.6	2.1		1.3	2.0				
Corosolic acid			3.0	1.4	1.5		1.1	2.2				
Other triterpene		6.4	1.7	1.7	2.3		2.8	2.3	0.4	0.3		
Total triterpenes		**15.4**	**63.9**	**60.2**	**70.9**	**3.6**	**89.2**	**68.6**	**0.5**	**0.5**	**41.5**	
C34 alky ester	C34								0.6	0.4		
C36 alkyl ester	C36	0.6							0.5	0.3		
C38 alkl ester	C38	0.3							0.3	0		
C40 alkyl ester	C40	0.7							0.5	0		
Other wax esters		5.2										
Total wax esters		**6.8**							**1.9**	**0.7**		
Not identified		**2.8**	**8.6**	**5.1**	**11.8**	**5.3**	**6.4**	**10.5**	**7.0**	**6.9**	**10.3**	**25.7**

Note: Data is presented as percent of total wax based on integration of GC-FID chromatograms. Identity of components was confirmed by GC-MS. Cherry samples are explained in detail in results.TR indicates trace amount below level of accurate quantification.

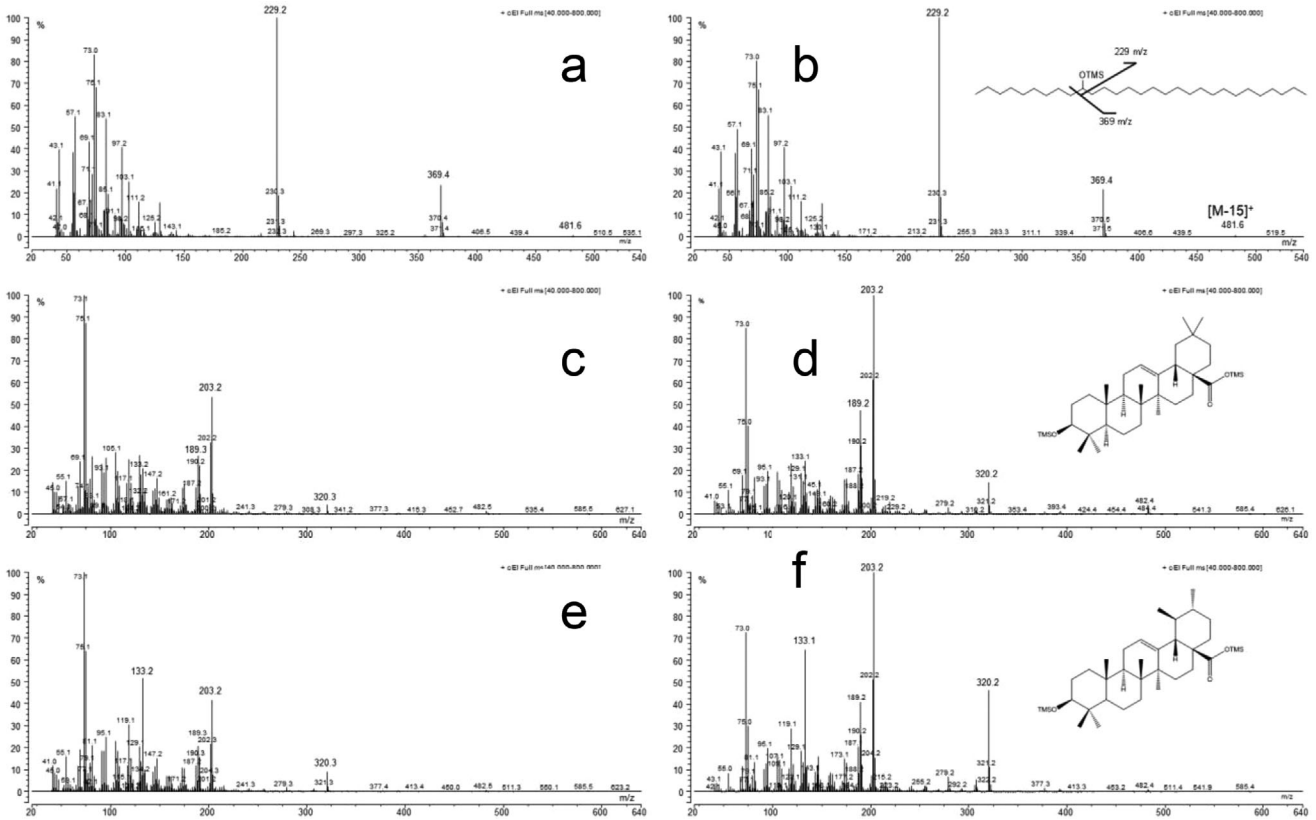


FIGURE 2 | Mass spectra of detected compounds and standards. (a) Spectrum of Peak 17 from Saskatoon berry, (b) mass spectrum of nonacosan-10-ol from Colorado blue spruce, (c) mass spectrum of Peak 26 from Saskatoon berry, (d) mass spectrum of oleanolic acid standard, (e) mass spectrum of Peak 28 from Saskatoon berry, (f) mass spectrum of ursolic acid standard.

was washed for 10 s with $CHCl_3$, followed by a second $CHCl_3$ wash of 60 s. All washes were evaporated to dryness under a gentle flow of N_2 gas and weighed (Table S1). Total wax ranged from 0.09 mg/g cherry for Sample C to 0.11 mg/g cherry for Sample B. Average wax load was calculated as 9.15 ± 0.90 μg/mm^2 cherry surface area. Taking into consideration the 70 s total wash time, wax recovered in the first 10 s wash ranged from 78.9% for Sample B to 87.8% for Sample C. For all samples, the $CHCl_3$ remained clear, without red coloring, after 70 s of washing suggesting that the fruit cuticle remained intact.

The chemical composition of the cherry wax Samples A and B (10 s washes) was determined by gas chromatography with mass spectrometry (GC/MS) following silylation with BSTFA in pyridine. Figure 3b shows the GC/FID chromatogram obtained from sample B, with numbering of the major peaks (Figure 3c). Three main components were observed with additional compounds at lower abundance. A total of 16 components were identified and GC/MS spectra are shown in Supporting Information S2. The major wax component present in the chloroform soluble wax was identified as the pentacyclic triterpene ursolic acid, accounting for 42%–47% (Table 1) of the total extracted wax (Figure 3c, Peak 14). The two additional abundant components of the wax were the C29 alkane nonacosane (Figure 3c, Peak 5, 21%–23%) and a second triterpene, oleanolic acid (Figure 3c, Peak 12, 11%–13%). Other components of the wax were identified by retention time as C27, C28, C30, and C31 *n*-alkanes. Mass spectra of these components showed characteristic fragmentation for alkanes, but the molecular ions $[M]^+$ could not always be detected. Two free fatty acids, hexadecanoic acid (palmitic acid, 16:0) and octadecanoic acid (stearic acid, 18:0) were identified from their TMS-esters and accounted for 0.5%–0.6% of total extracted wax. Primary alcohols were not observed, but Peak 7 showed a mass spectrum characteristic of the secondary alcohol nonacosan-10-ol. Small fragment ions at 243.3 and 355.4 *m/z* suggested the presence of low levels of the isomer nonacosan-11-ol. Levels of secondary alcohol varied between samples, accounting for 2.8% of total wax in Sample B, but only 0.1% in Sample A. Five additional peaks with retention time close to the two major triterpenes were tentatively identified as other triterpenes. Peaks 15 and 16 (Figure 4) were tentatively identified as the TMS derivatives of maslinic acid ((2α,3β)-2,3-dihydroxy-olean-12-en-28-oic acid) and corosolic acid ((2α,3β)-2,3-dihydroxy-urs-12-en-28-oic acid) by comparison to published GC-MS spectra [36, 38–40]. Due to their low abundance and the lack of additional material, a conclusive identification of these compounds was not possible in this study. Other minor components that could not be confidently identified accounted for between 5% and 8.5% of the total wax in Samples A and B. For Sample C, the dried wax from the 10 s wash was washed with hexane at room temperature (23°C) and the hexane was removed to a fresh vial (hexane fraction) and the remaining wax was then dissolved in $CHCl_3$ ($CHCl_3$ fraction). GC analysis showed that the hexane wash preferentially extracted the alkanes and secondary alcohol from the wax with the total alkane content raising to 84.6% while the $CHCl_3$ fraction was enriched in triterpenes (Table 1). Comparison of 10 s wash with 60 s wash

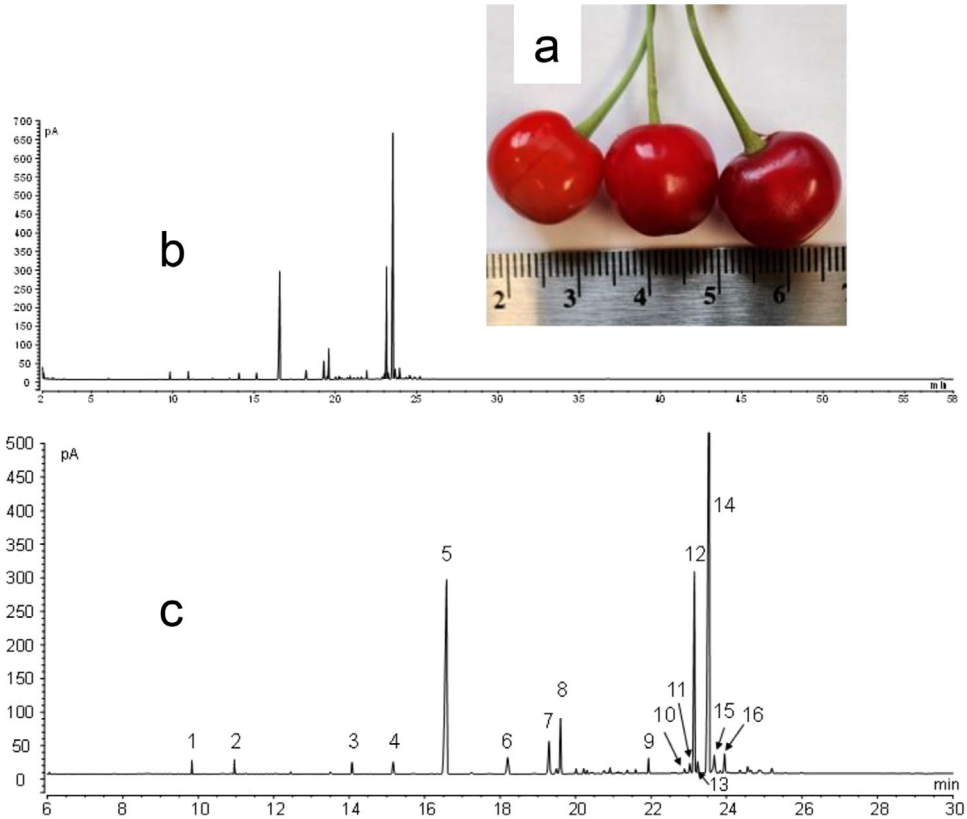


FIGURE 3 | (a) Canadian dwarf sour cherries showing glossy appearance, (b) full chromatogram of dwarf sour cherry wax extract, (c) enlarged chromatogram of 6–30 min showing major peaks. Identities of labeled peaks: (1) hexadecanoic acid, (2) octadecanoic acid, (3) heptacosane, (4) octacosane, (5) nonacosane, (6) triacontane, (7) nonacosan-10-ol, (8) hentriacontane, (9) tricontanoic acid, (10) triterpene, (11) triterpene, (12) oleanolic acid, (13) triterpene, (14) ursolic acid, (15) maslinic acid, and (16) corosolic acid.

samples also showed a relative enrichment of triterpenes in the longer wash with a corresponding reduction in alkanes.

3.3 | Haskap

Ripe haskap berries were collected from two different bushes, selected by differences in appearance of the berries (Figure 5a,c) and were designated Haskap 1 and 2, respectively. Berries from both bushes had an obvious wax bloom on the fruit surfaces. Wax was collected by gently agitating batches of berries in $CHCl_3$ for 30 s. Fruit size, surface area, and amount of wax collected was not measured in this exploratory study. After silylation of the samples, components were separated by GC with FID detection for quantification. For Haskap 1, 36 components were identified by GC-MS (Figure 3b and Supporting Information S3) and quantified (Table 1). The major component (Figure 5b, Peak 15), accounting for 52.3% of the total wax was identified as the secondary alcohol nonacosan-10-ol. The two other abundant components of the wax were the C29 secondary alkane-diol nonacosan-5,10-diol (Peak 23, 11.4%) and the C29 alkane nonacosane (Peak 9, 9.7%). Other secondary alkane-diols and odd-chain alkanes were also detected with total secondary alkane-diols and alkanes making up 15.4% and 13% of the total wax respectively. For the secondary alkane-diols, characteristic fragment ions in the EI mass spectra resulting from fragmentation α-to the -OTMS functional group [41, 42] enabled confident location of the hydroxyl groups, and determination of chain length (Figure 6). Free fatty acids (1.2%) and triterpenes (0.5%) were of low abundance with α-amyrin being the only triterpenoid identified with confidence. For Haskap 1, primary alcohols accounted for 8.2% of the wax, with chain lengths from C22 to C30. The C28 alcohol octacosan-1-ol was most abundant at 2.7%. Alkyl esters made up 1.9% of the wax and 7% of the wax was composed of unidentified components. The Haskap 2 sample (Figure 5d) had a similar composition to Haskap 1, but the GC-FIG chromatogram showed that some components had much lower peak areas. These peaks were identified as primary alcohols, with total primary alcohol levels being reduced to 1.9%, of which triacontanol (C30) made up 1.2%.

3.4 | Red twinberry

A single sample of 65.2 g of ripe red twinberries (Figure 7a) was collected. A 10 s $CHCl_3$ wash of the berries yielded 58.6 mg of wax. Separation of wax components with FID detection revealed a relatively simple profile, with GC-MS analysis enabling the identification of 16 components (Figure 7c, Supporting Information S4 and Table 1). In contrast to haskap, triterpenes were abundant, with the triterpenoid ursolic acid (Figure 7c, Peak 17) being the dominant component of the wax at 31.5% of total wax. Ursolic acid was accompanied by oleanolic acid (6.4%) and betulinic acid (3.6%). The second most abundant component of the wax was nonacosan-10-ol at 24.4%. Hepracosan-9-ol was also detected as a minor component (0.6%). Alkanes accounted for 11.5% of the wax with nonacosane at 10.4% and low levels of heptacosane (0.6%)

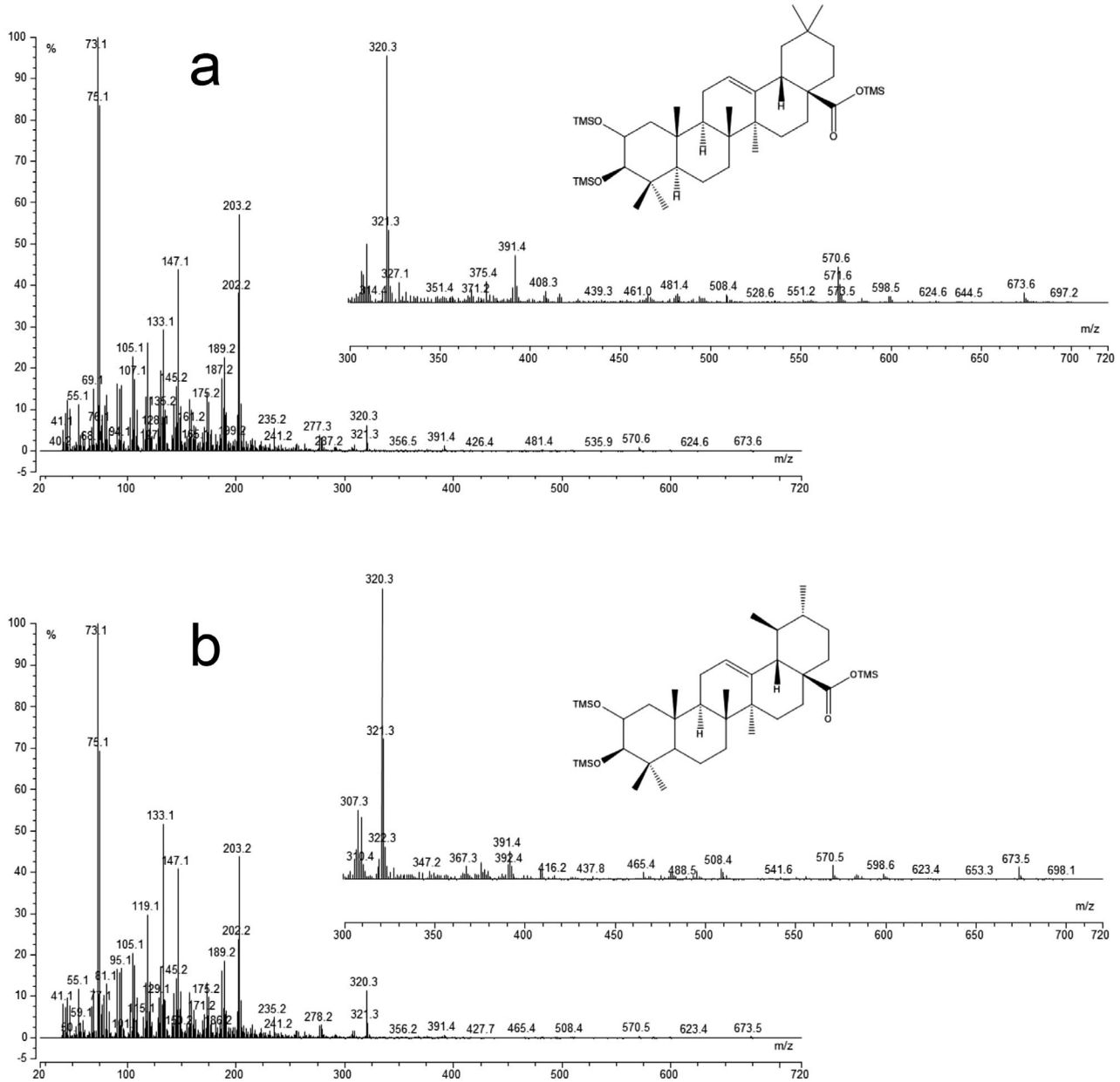


FIGURE 4 | Mass spectra obtained from dwarf sour cherry wax Peaks 15 and 16, tentatively identified as (a) maslinic acid and (b) corosolic acid.

and hentriacontane (0.5%). C22 (docosanoic acid, 0.3%) and C24 (tetracosanoic acid, 1.3%) saturated fatty acids were detected together with three primary alcohols, tetracosane-1-ol (2.7%), hexacosan-1-ol (3%), and octacosan-1-ol (2.2%). The wax also contained three C29 secondary alkane-diols with unidentified components making up 10.3% of the wax. Alkyl esters were not an obvious component of the wax.

3.5 | Colorado Blue Spruce

Although the wax from blue spruce shoots (Figure 8a) was originally collected as a source of nonacosan-10-ol, a brief survey of the other major components of the wax was conducted. The GC-FID chromatogram from the sample is shown in Figure 8a,b, and 9 components from the 11 highest peaks were identified from their mass spectra (Figure 8c, Supporting Information S5 and Table 1) accounting for 74.3% of the total wax. Nonacosan-10-ol comprised 53% of the total wax and this was accompanied by a small amount (0.3%) of the C27 secondary alcohol heptacosan-10-ol (Figure 8c, Peak 3). Four C29 alkane diols were detected nonacosan-10,13-diol, nonacosan-7,10-diol, nonacosan-5,10-diol, and nonacosan-4,10-diol. The remaining identified components were fatty acids (24:0 and 30:0) and the diterpenoid abietic acid (abieta-7,13-dien-18-oic acid). No primary alcohols or alkanes could be detected.

4 | Discussion

The purpose of the work was to survey the major surface wax components of ripe fruit of the selected species. Percentage

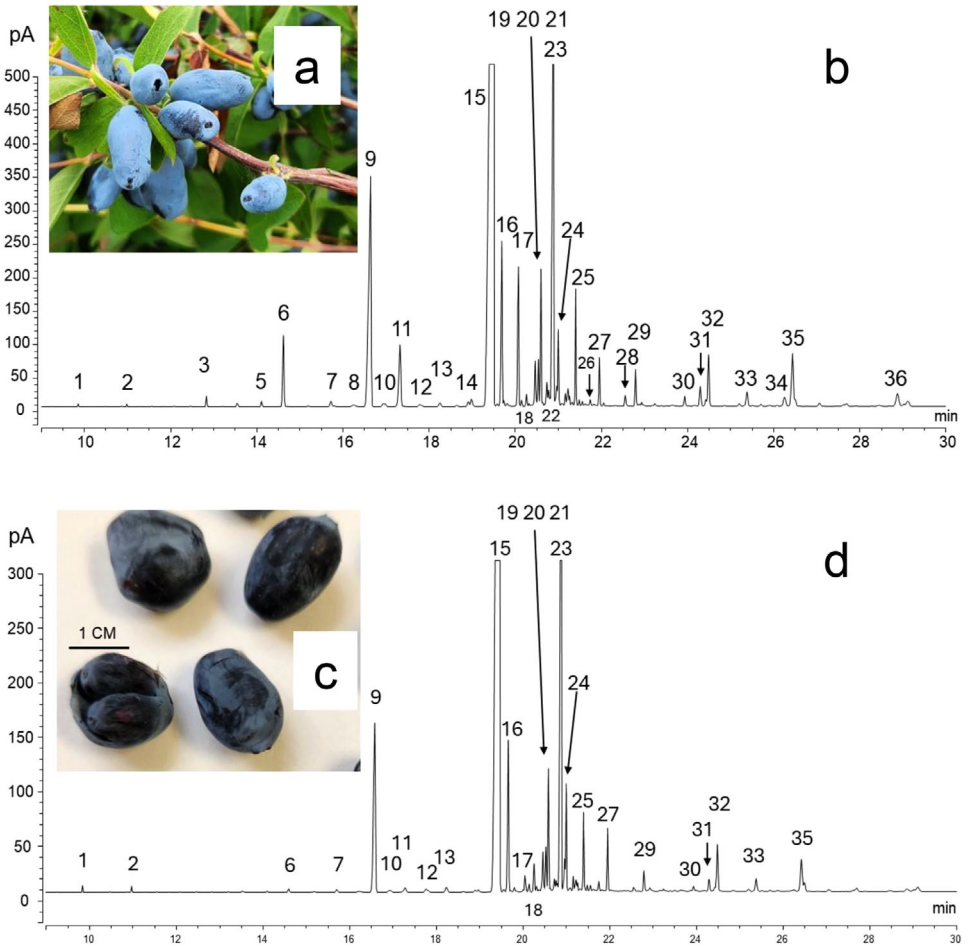


FIGURE 5 | (a) Fruit of haskap 1 photographed in daylight, (b) enlarged chromatogram of haskap 1 wax separation for 6–30 min showing major peaks, (c) fruit of haskap 2, photographed under laboratory lighting and showing partly exposed fruit and reduced bracteolar copula, (d) enlarged chromatogram of haskap 2 wax separation for 6–30 min showing major peaks. Identities of labelled peaks, (1) hexadecanoic acid, (2) octadecanoic acid, (3) docosan-1-ol, (4) not identified, (5) heptacosane, (6) tetracosan-1-ol, (7) tetracosanoic acid, (8) heptacosan-9-ol, (9) nonacosane, (10) unknown, (11) hexacosan-1-ol, (12) octacosan-10-ol, (13) triacontane, (14) hexacosanoic acid, (15) nonacosan-10-ol, (16) hentriacontane, (17) octacosan-1-ol, (18) triacontane-10-ol, (19) nonacosan-10,13-diol, (20) nonacosan-8,11 diol, (21) nonacosan-7,10-diol, (22) nonacosen-10-ol, (23) nonacosan-5,10-diol, (24) nonacosan-4,9-diol, (25) triacontan-1-ol, (26) α-amyrin, (27) tricontanoic acid, (28) triterpene, (29) C34 alkyl ester, (30) triterpene, (31) C36 alkyl ester, (32) unknown, (33) unknown, (34) C38 alkyl ester, (35) unknown, and (36) C40 alkyl ester.

composition is based on the area percent of peaks from FID chromatograms of wax components separated by GC and may not fully represent the components by percentage weight. As the constituents show variation in chemical structure, a more comprehensive quantitative analysis would need to consider FID response factors and would require multiple standards for the construction of calibration curves. The data presented here describes the major components of the waxes and can be used for comparative studies, and a starting point for screening of chemical diversity. For compound identification, we did not rely only on computer generated matches to mass spectral libraries. Spectra were individually examined and compared to published spectra, or standards where available. Careful attention to retention times and the lower *m/z* fragment ions aided in identification of molecules lacking strong signals for diagnostic ions, such as the very-long-chain alkanes (57, 71, 85, 99 *m/z* series) and aldehydes (57, 69, 82, 96 *m/z* series) in Saskatoon berry. For these molecules, a softer ionization method such as collision induced (CI) ionization may be more appropriate rather than the hard ionization of EI. As many analyses do not publish mass spectra, spectra collected from all numbered peaks in this study have been included in supplemental figures to aid with future work. All samples contain additional components present at very low levels. These could not be identified with confidence using the approach described in this report.

All fruit were collected when ripe, at the stage at which they would be harvested for consumption or processing. Wax was extracted from freshly collected fruit immediately after the fruit was picked as it has been reported that active synthesis of wax continues after fruit harvest, influencing both amount and composition, [3, 16, 17]. Wax samples were collected by washing with $CHCl_3$ as this solvent has previously been reported as suitable [9]. Samples were resuspended in $CHCl_3$, after evaporation and concentration, and transferred to glass GC vial inserts for analysis. Samples were silylated to aid in identification of alkyl compounds containing hydroxyl and carboxyl groups [43]. This was conducted directly in the GC vial inserts using pyridine

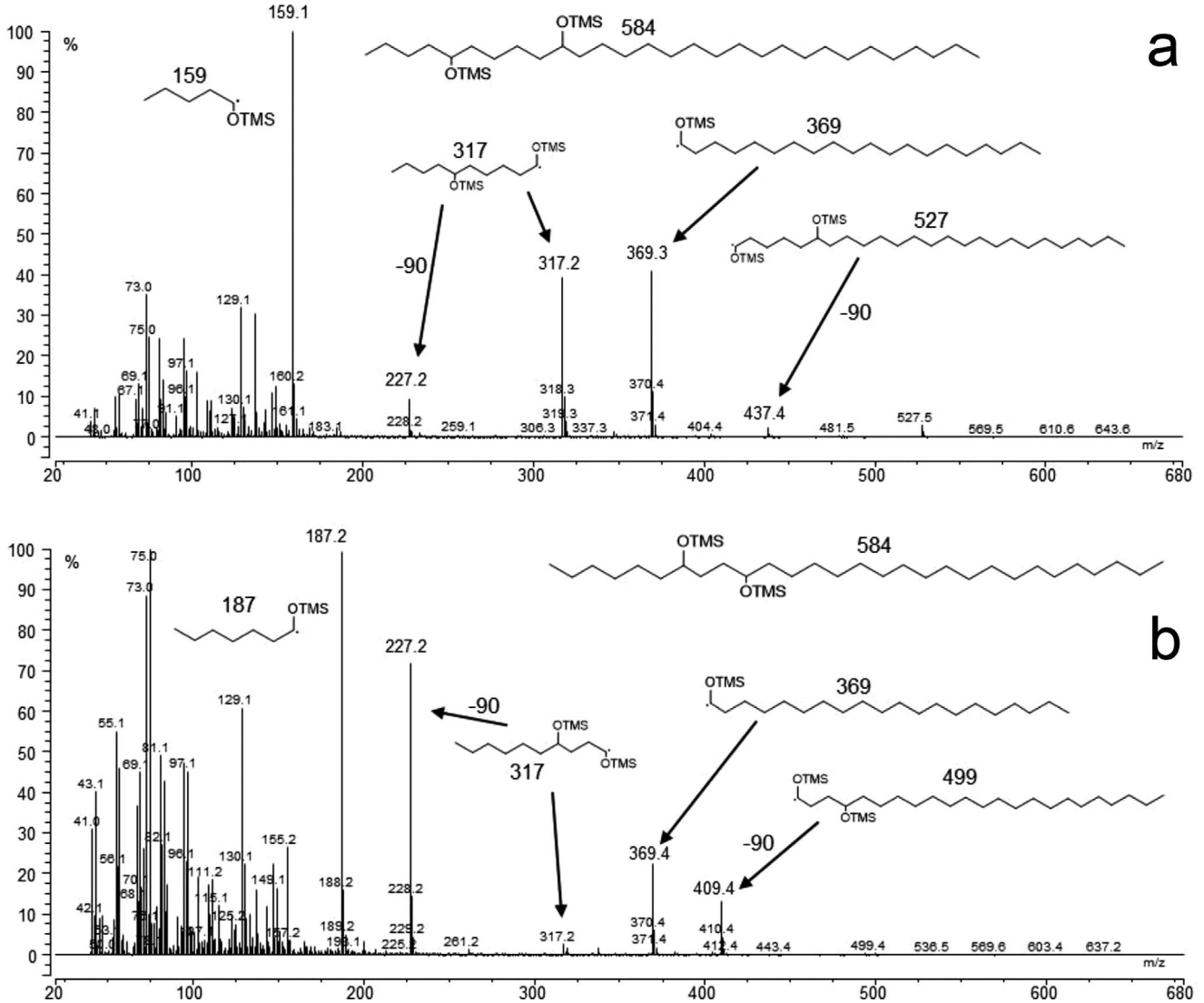


FIGURE 6 | Mass spectra of secondary diols, Peaks 23 and 21 from haskap showing patterns of fragmentation. (a) nonacosan-5,10-diol, (b) nonacosan-7,10-diol.

as solvent. The pyridine and residual silylating agent were not removed prior to sample injection. Resuspending samples in nonpolar solvent alone, such as hexane, a commonly used solvent carrier for GC analysis, was avoided. As shown for the dwarf sour cherry samples, resuspending, or washing with hexane could enrich the hydrocarbon content of the sample at the expense of the less soluble triterpenoids.

The composition of Saskatoon berry wax was close to that previously reported for the variety "Smokey" [23]. The predominant component, nonacosan-10-ol is found in a wide variety of plant waxes, often as the major component [10]. Some additional components were observed including free fatty acids ranging in length from C16 to C30 and triterpenes. These may have been overlooked previously. Canadian dwarf sour cherry wax was rich in pentacyclic triterpenoids, with ursolic acid being the major component and nonacosan-10-ol only present at low levels. Oleanolic and ursolic acid derivatives maslinic and corosolic acid were tentatively identified as minor components. The wax composition of Canadian dwarf sour cherry fruit appeared similar to that reported previously for sweet cherry where the major components were identified as ursolic acid and nonacosane [16, 27]. For Canadian dwarf sour cherry, differences in percentage composition of lipid classes observed between first and second extractions of the fruit suggest that the wax components may not be evenly distributed within the epicuticular and intracuticular wax layers, with alkanes perhaps showing enrichment in the epicuticular wax. More detailed study using mechanical methods to remove epicuticular wax, such as gum arabic polymer would be required to confirm this observation [44].

Only limited information is available concerning the wax composition of haskap berries. A study of the triterpenoid composition of leaf and fruit wax from a single variety, *L. caerulea* var *kamtschatica*, reported triterpenoids as low abundance components accounting for 6.5% of a fruit wax [32]. These were dominated by tetracyclic triterpenoids, primarily sitosterol, with Hop-22(29)-en-3-one, α-amyrin β-amyrin, and ursolic acid as pentacyclic triterpenoids. In our study, only α-amyrin could be identified with confidence. As triterpenoids were not separated

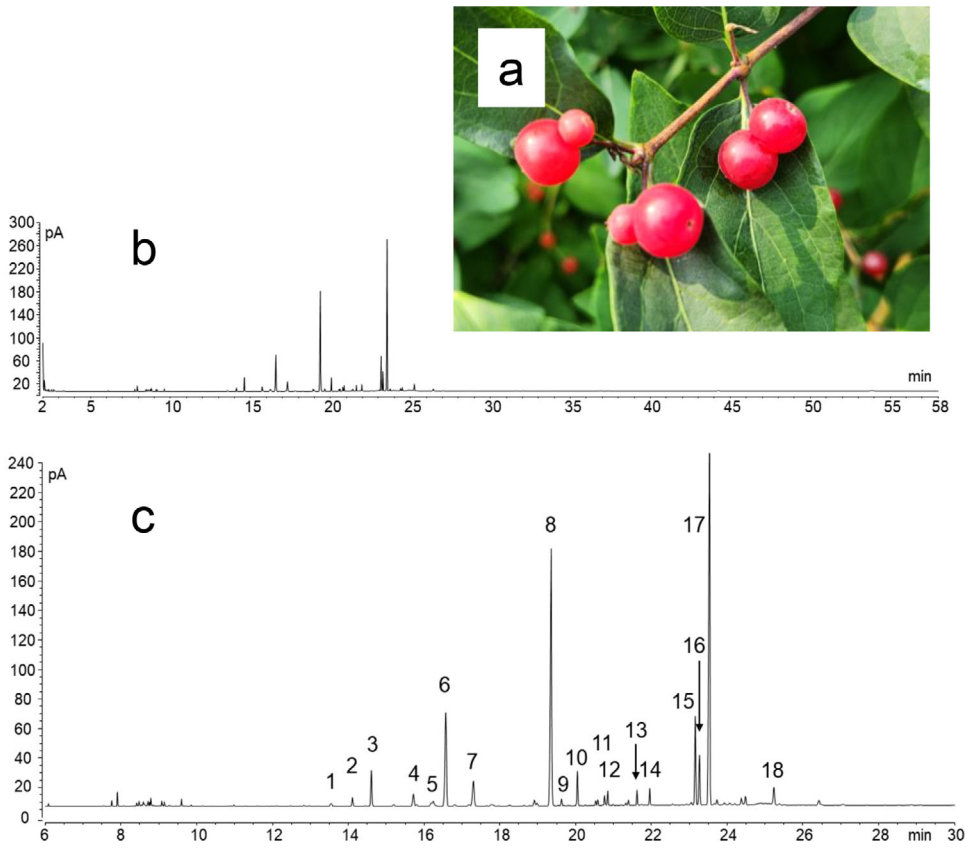


FIGURE 7 | (a) Red twinberry, (b) full chromatogram of red twinberry wax extract, and (c) enlarged chromatogram of 6–30 min showing major peaks. Identities of labelled peaks: (1) docosanoic acid (2) heptacosane, (3) tetracosane-1-ol, (4) tetracosanoic acid, (5) heptacosan-9-ol, (6) nonacosane, (7) hexacosan-1-ol, (8) nonacosan-10-ol, (9) hentriacontane, (10) octacosan-1-ol, (11) nonacosan-8,11-diol, (12) nonacosan-7,10-diol, (13) nonacosan-5,10-diol, (14) unknown, (15) oleanolic acid, (16) betulinic acid, (17) ursolic acid, and (18) unknown.

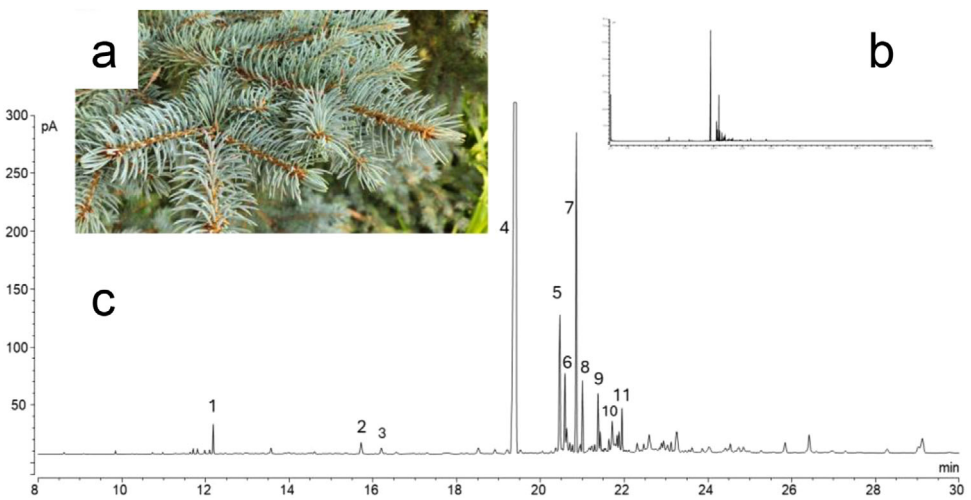


FIGURE 8 | (a) Colorado blue spruce shoots, (b) full chromatogram blue spruce wax extract, and (c) enlarged chromatogram of 6–30 min showing major peaks. Identities of labeled peaks: (a) abietic acid, tetracosanoic acid, (3) heptacosan-10-ol, (4) nonacosan-10-ol, (5) nonacosan-10,13-diol, (6) nonacosan-7,10-diol, (7) nonacosan-5,10-diol, (8) nonacosan-4,10-diol, (9) unknown, (10) unknown, (11) tricontanoic acid.

from other components, it is likely that additional, low abundance triterpenoids were present, but not identified. A more recent report, characterizing the wax from three different haskap varieties reported the identification of 158 chemical components [17]. Although also using chloroform extraction and GC-MS analysis, the composition of wax in our study has little in common with that report. Major alkanes components were reported as being 7,9-dimethylhexadecane, 2,6,11-trimethyldodecane, and

nonadecane with a C18 ketone as the most abundant wax component. From our analysis of berries collected in Canada, it is clear that nonacosan-10-ol is the major wax component. This is accompanied by the secondary diol nonacosan-5,10-diol as the second most abundant giving the wax a high content of oxygenated aliphatic components. Similar to blueberries, ripe haskap have a substantial wax bloom on the surface. The wax layer imparts a blue coloration when viewed in daylight, although wax removal reveals a darker purple color below the wax (Figure 1a). Haskap and blueberry do not, however, share the same wax composition, with blueberry wax containing abundant triterpenoids, primarily ursolic acid and oleanolic acid, and β-diketones, primarily hentriacontane-10,12-dione [4]. The mechanism of light scattering by haskap surface wax may have more in common with that of the wax of blue spruce, as both are rich in nonacosan-10-ol. The wax of the second *Lonicera* species examined, L. *utahensis*, contained ursolic acid as the most abundant component and ripe berries had a more glossy appearance, illustrating wax diversity within a genus, as noted previously for triterpenoid distribution in leaf wax [8].

The potential health promoting properties of fruit plays an important part in many marketing strategies and attention is often drawn to presence of high concentrations of compounds such as anthocyanins with demonstrated antioxidant properties [45]. These compounds also have importance in determining potential uses and value of fruit by-products such as pomace [46]. Relatively little attention has been given to the potential value of waxes from berries. Although VLC-primary alcohols such as hexacosan-1-ol and octacosan-1-ol are components of policosanol, a nutraceutical product derived from waxes of plants such as sugar cane and rice bran [47], the potential health promoting activities of VLC-secondary alcohols such as nonacosan-10-ol are unknown. Nonacosan-10-ol from forestry waste has; however, been shown to have application as a renewable highly-hydrophobic coating material [48]. VLC-alkanes in food, such as nonacosane likely have limited solubility and uptake but may become available for metabolic processes after ω-oxidation [49]. In contrast, there are many health claims associated with triterpenoids such as ursolic acid the major component of Canadian dwarf sour cherry wax [50–52]. Triterpenoids are also considered to play an important role in biotic interactions between plants and other organisms such as insect pests and pathogens [53].

Surface waxes play a role in berry shelf life and consumer appeal and are potential targets for breeding of improved varieties. Characterizing wax composition will aid in obtaining a better understanding of the functional contribution of wax components in processes such as control of water loss pre- and post-harvest, and the role of wax in interactions with fruit pests and fungal pathogens. An extension of this study would be to determine the relative contributions of the wax bloom, and waxes embedded within the fruit cuticle to these properties. The data from cherry presented here suggests that chemical components are likely not evenly distributed between cuticular and epicuticular layers. The ability to rapidly screen for wax diversity will be an important aspect of breeding for improved varieties. The GC-FID method applied in this study offers a simple and sensitive method for screening breeding lines. Although not an objective of the pilot study, there is clearly evidence of wax compositional diversity in haskap and more detailed analysis of diversity collections would be of great interest. In addition, analysis of wax composition during fruit development, the effect of growing environment and post-harvest changes would also be of value for all of these species.

Author Contributions

M.A.S. and H.Z. did the project conceptualization. M.A.S. and H.Z. performed sample identification and collection. Wax extraction was done by M.A.S. M.A.S. and D.H. did the GC/FID and GC/MS, compound identification and quantification. Figures and manuscript writing was done by M.A.S. and H.Z. All authors read and approved the manuscript.

Acknowledgment

The authors thank Dr. Bob Bors for helping identifying the variety of sour cherry. Haixia Zhang thanks the Saskatchewan Agriculture Development Fund (ADF 20240596) for funding towards this work.

Open access funding provided by the Gouvernement du Canada Agriculture et Agroalimentaire Canada library.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

1. I. Lara, B. Belge, and L. F. Goulao, "A Focus on the Biosynthesis and Composition of Cuticle in Fruits," *Journal of Agricultural and Food Chemistry* 63 (2015): 4005–4019, https://doi.org/10.1021/acs.jafc.5b00013.

2. I. Lara, A. Heredia, and E. Dominguez, "Shelf Life Potential and the Fruit Cuticle: The Unexpected Player," *Frontiers in Plant Science* 10 (2019): 770, https://doi.org/10.3389/fpls.2019.00770.

3. R. Fernandez-Munoz, A. Heredia, and E. Dominguez, "The Role of Cuticle in Fruit Shelf Life," *Current Opinion in Biotechnology* 78 (2022): 102802, https://doi.org/10.1016/j.copbio.2022.102802.

4. Y. Yan, M. Dossett, and S. D. Castellarin, "Cuticular Waxes Affect Fruit Surface Color in Blueberries," *Plants People Planet* 5 (2023): 736–751, https://doi.org/10.1002/ppp3.10368.

5. R. Middleton, S. A. Tunstad, A. Knapp, S. Winters, S. McCallum, and H. Whitney, "Self-Assembled, Disordered Structural Color From Fruit Wax Bloom," *Science Advances* 10 (2024): adk4219, https://doi.org/10.1126/sciadv.adk4219.

6. C. M. Asensio, M. L. Arpaia, and D. Obenland, "The Role of Fruit Surface Bloom in Consumer Preference for Blueberries: Sensory Evaluation and Multisensory Interactions," *Foods* 14 (2025): 455, https://doi.org/10.3390/foods14030455.

7. R. Liu, L. Zhang, S. Xiao, et al., "Ursolic Acid, the Main Component of Blueberry Cuticular Wax, Inhibits *Botrytis cinerea* Growth by Damaging Cell Membrane Integrity," *Food Chemistry* 415 (2023): 135753, https://doi.org/10.1016/j.foodchem.2023.135753.

8. L. T. D. Nguyen, N. Groth, K. Mondloch, E. B. Cahoon, K. Jones, and L. Busta, "Project ChemicalBlooms: Collaborating With Citizen Scientists to Survey the Chemical Diversity and Phylogenetic Distribution of Plant Epicuticular Wax Blooms," *Plant Direct* 8 (2024): 588, https://doi.org/10.1002/pld3.588.

9. R. Jetter, L. Kunst, and A. L. Samuels, "Composition of Plant Cuticular waxes," in *Annual Plant Reviews Volume 23: Biology of the Plant Cuticle*, ed. M. Riederer and C. Muller (Blackwell Publications, 2006), 145–181.

10. L. Busta and R. Jetter, "Moving Beyond the Ubiquitous: The Diversity and Biosynthesis of Specialty Compounds in Plant Cuticular Waxes," *Phytochemistry Reviews* 17 (2018): 1275–1304, https://doi.org/10.1007/s11101-017-9542-0.

11. P. Trivedi, N. Nguyen, A. L. Hykkerud, et al., "Developmental and Environmental Regulation of Cuticular Wax Biosynthesis in Fleshy Fruits," *Frontiers in Plant Science* 10 (2019): 431, https://doi.org/10.3389/fpls.2019.00431.

12. M. Lewandowska, A. Keyl, and I. Feussner, "Wax Biosynthesis in Response to Danger: Its Regulation Upon Abiotic and Biotic Stress," *New Phytologist* 227 (2020): 698–713, https://doi.org/10.1111/nph.16571.

13. D. Hegebarth and R. Jetter, "Cuticular Waxes of *Arabidopsis thaliana* Shoots: Cell-Type-Specific Composition and Biosynthesis," *Plants* 6 (2017): 27, https://doi.org/10.3390/plants6030027.

14. X. Zhao, L. Huang, L. Kang, et al., "Comparative Analyses of Cuticular Waxes on Various Organs of Faba Bean (*Vicia faba* L.)," *Plant Physiology and Biochemistry* 139 (2019): 102–112, https://doi.org/10.1016/j.plaphy.2019.03.015.

15. W. Wu, B. Jiang, R. Liu, et al., "Structures and Function of Cuticular Wax in Postharvest Fruit and Its Regulation: A Comprehensive Review With Future Perspectives," *Engineering* 23 (2023): 118–129, https://doi.org/10.1016/j.eng.2022.12.006.

16. B. Belge, M. Llovera, E. Comabella, et al., "Characterization of Cuticle Composition After Cold Storage of 'Celeste' and 'Somerset' Sweet Cherry Fruit," *Journal of Agricultural and Food Chemistry* 62 (2014): 8722–8729, https://doi.org/10.1021/jf502650t.

17. Z. Peng, H. Chen, B. Niu, et al., "Composition and Micromorphological Determination of Blue Honeysuckle Fruit (*Lonicera caerulea* L.) Cuticular Wax and Its Effects on Fruit Post-Harvest Quality," *Food Chemistry* 463 (2025): 141330, https://doi.org/10.1016/j.foodchem.2024.141330.

18. S. Williams and B. Bors, *Growing Fruit in Northern Gardens* (Coteau Books, 2017), 312.

19. G. Mazza, "Compositional and Functional Properties of Saskatoon Berry and Blueberry," *International Journal of Fruit Science* 5 (2005): 101–120, https://doi.org/10.1300/J492v05n03_10.

20. J. Fang, "Nutritional Composition of Saskatoon Berries: A Review," *Botany* 99 (2020): 175–184, https://doi.org/10.1139/cjb-2019-0191.

21. C. Kodikara, N. Bandara, T. Netticadan, and C. Wijekoon, "Canadian Prairie Berries: Bioactive Compounds and Their Potential Health Benefits," *Food Reviews International* 40 (2024): 2486–2513, https://doi.org/10.1080/87559129.2023.2273934.

22. D. Li, Z. Cheng, W. Z. W. Ibadullah, R. Shukri, Q. Duan, and N. A. Mustapha, "Saskatoon Berry (*Amelanchier alnifolia*): Nutritional Composition, Phytochemical, Health Benefits, Shelf-Life Extension and Utilization," *Food Science and Biotechnology* 35 (2025): 1097–1114, https://doi.org/10.1007/s10068-025-01997-7.

23. L. O. Knowles, N. R. Knowles, and J. Tewari, "Aliphatic Components of the Epicuticular Wax of Developing Saskatoon (*Amelanchier alnifolia*) Fruit," *Canadian Journal of Botany* 74 (1996): 1260–1264, https://doi.org/10.1139/b96-152.

24. F. Blando and B. D. Oomah, "Sweet and Sour Cherries: Origin, Distribution, Nutritional Composition and Health Benefits," *Trends in Food Science & Technology* 86 (2019): 517–529, https://doi.org/10.1016/j.tifs.2019.02.052.

25. A. Sokol-Letowska, A. Z. Kucharska, G. Hodun, and M. Golba, "Chemical Composition of 21 Cultivars of Sour Cherry (*Prunus cerasus*) Fruit Cultivated in Poland," *Molecules* 25 (2020): 4587, https://doi.org/10.3390/molecules25194587.

26. K. S. Markley and C. E. Sando, "The Wax-Like Constituents of the Cuticle of the Cherry, *Prunus avium* L.," *Journal of Biological Chemistry* 119 (1937): 641–645, https://doi.org/10.1016/S0021-9258(18)74410-4.

27. S. Peschel, R. Franke, L. Schreiber, and M. Knoche, "Composition of the Cuticle of Developing Sweet Cherry Fruit," *Phytochem* 68 (2007): 1017–1025, https://doi.org/10.1016/j.phytochem.2007.01.008.

28. E. M. Gerbrandt, R. H. Bors, and R. N. Chibbar, "Agronomic Potential of Fruit Size and Yield Traits in Blue Honeysuckle (*Lonicera caerulea* L.) Foundation Germplasm," *Euphytica* 214 (2018): 107, https://doi.org/10.1007/s10681-018-2184-5.

29. G. B. Celli, A. Ghanem, and M. S. L. Brooks, "Haskap Berries (*Lonicera caerulea* L.)—A Critical Review of Antioxidant Capacity and Health Related Studies for Potential Value-Added Products," *Food Processing Technology* 7 (2014): 1541–1554.

30. R. Becker and A. Szakiel, "Phytochemical Characteristics and Potential Therapeutic Properties of Blue Honeysuckle *Lonicera caerulea* L. (*Caprifoliaceae*)," *Journal of Herbal Medicine* 16 (2019): 100237, https://doi.org/10.1016/j.hermed.2018.10.002.

31. M. Golba, A. Sokol-Letowska, and A. Z. Kucharska, "Health Properties and Composition of Honeysuckle Berry *Lonicera caerulea* L. An Update on Recent Studies," *Molecules* 25 (2020): 749, https://doi.org/10.3390/molecules25030749.

32. R. Becker, C. Paczkowski, and A. Szakiel, "Triterpenoid Profile of Fruit and Leaf Cuticular Waxes of Edible Honeysuckle *Lonicera caerulea* Var. *Kamtschatica*," *Acta Societatis Botanicorum Poloniae* 86 (2017): 3539, https://doi.org/10.5586/asbp.3539.

33. M. Turner and E. Kuhlmann, *Trees and Shrubs of the Pacific Northwest* (Timber Press, 2014), 448.

34. S. R. Whitehead and M. D. Bowers, "Iridoid and Secoiridoid Glycosides in a Hybrid Complex of Bush Honeysuckles (*Lonicera* spp., *Caprifoliaceae*): Implications for Evolutionary Ecology and Invasion Biology," *Phytochemistry* 86 (2013): 57–63, https://doi.org/10.1016/j.phytochem.2012.10.012.

35. R. Jetter and M. Riederer, "Epicuticular Crystals of Nonacosan-10-ol: *In Vitro* Reconstitution and Factors Influencing Crystal Habits," *Planta* 195 (1994): 257–270, https://doi.org/10.1007/BF00199686.

36. M. I. Razborsek, D. B. Voncina, V. Dolecek, and E. Voncina, "Determination of Oleanolic, Betulinic and Ursolic Acid in *Lamiaceae* and Mass Spectral Fragmentation of Their Trimethysilylated Derivatives," *Chromatographia* 67 (2008): 433–440, https://doi.org/10.1365/s10337-008-0533-6.

37. J. Pollier and A. Goossens, "Oleanolic Acid," *Phytochemistry* 77 (2012): 10–15, https://doi.org/10.1016/j.phytochem.2011.12.022.

38. A. Caligiani, G. Malavasi, G. Palla, A. Marseglia, M. Tognolini, and R. Bruni, "A Simple GC-MS Method for the Screening of Betulinic, Corosolic, Maslinic, Oleanolic and Ursolic Acid Contents in Commercial Botanicals Used as Food Supplement Ingredients," *Food Chemistry* 136 (2013): 735–741, https://doi.org/10.1016/j.foodchem.2012.08.011.

39. M. Wiebel, L. Wende, K. Bensberg, T. Zschau, and S. F. Kirsch, "Development of a Gas Chromatography-Mass Spectrometry (GC-MS) Method for the Characterization and Quantification of Triterpenic Acids," *Food Chemistry* 493 (2025): 146012, https://doi.org/10.1016/j.foodchem.2025.146012.

40. A. Lourenco, A. V. Marques, and J. Gominho, "The Identification of New Triterpenoids in *Eucalyptus globulus* Wood," *Molecules* 26 (2021): 3495, https://doi.org/10.3390/molecules26123495.

41. R. A. Franich, A. P. Gowar, and J. K. Volkman, "Secondary Diols of *Pinus radiata* Needle Epicuticular Wax," *Phytochemistry* 18 (1979): 1563–1564, https://doi.org/10.1016/S0031-9422(00)98497-8.

42. R. Jetter, M. Riederer, A. Seyer, and C. Mioskowski, "Homologous Long-Chain Alkanediols From Papaver Leaf Cuticular Waxes," *Phytochemistry* 42 (1996): 1617–1620, https://doi.org/10.1016/0031-9422(96)00180-X.

43. D. J. Harvey and P. Vouros, "Mass Spectrometric Fragmentation of Trimethylsilyl and Related Alkysilyl Derivatives," *Mass Spectrometry Reviews* 39 (2020): 105–211, https://doi.org/10.1002/mas.21590.

44. R. Jetter and S. Schaffer, "Chemical Composition of the *Prunus laurocerasus* Leaf Surface. Dynamic Changes of the Epicuticulat Wax Film During Leaf Development," *Plant Physiology* 126 (2001): 1725–1737, https://doi.org/10.1104/pp.126.4.1725.

45. A. Szajdek and E. J. Borowska, "Bioactive Compounds and Health Promoting Properties of Berry Fruits: A Review," *Plant Foods for Human Nutrition* 63 (2008): 147–156, https://doi.org/10.1007/s11130-008-0097-5.

46. E. Raczkowska and S. Pawel, "Health-Promoting Properties and the Use of Fruit Pomace in the Food Industry—A Review," *Nutrients* 16 (2024): 2757, https://doi.org/10.3390/nu16162757.

47. J. Shen, F. Luo, and Q. Lin, "Policosanol: Extraction and Biological Functions," *Journal of Functional Foods* 57 (2019): 351–360, https://doi.org/10.1016/j.jff.2019.04.024.

48. C. R. McElroy, T. M. Attard, T. J. Farmer, et al., "Valorization of Spruce Needle Waste via Supercritical Extraction of Waxes and Facile Isolation of Nonacosan-10-ol," *Journal of Cleaner Production* 171 (2018): 557–566, https://doi.org/10.1016/j.jclepro.2017.10.002.

49. J. A. Barrowman, A. Rahman, M. B. Lindstrom, and B. Borgstrom, "Intestinal Absorption and Metabolism of Hydrocarbons," *Progress in Lipid Research* 28 (1989): 189–203, https://doi.org/10.1016/0163-7827(89)90012-X.

50. L. Wozniak, S. Skapska, and K. Marszalek, "Ursolic Acid—A Pentacyclic Triterpenoid With a Wide Spectrum of Pharmacological Activities," *Molecules* 20 (2015): 20614–20641, https://doi.org/10.3390/molecules201119721.

51. H. N. Nguyen, S. L. Ullevig, J. D. Short, L. Wang, Y. J. Ahn, and R. Asmis, "Ursolic Acid and Related Analogues: Triterpenoids With Broad Health Benefits," *Antioxidants* 10 (2021): 1161, https://doi.org/10.3390/antiox10081161.

52. R. Arulnangai, H. A. Thabassoom, H. V. Banu, K. Thirugnanasambandham, and R. Ganesamoorthy, "Recent Developments on Ursolic Acid and Its Potential Biological Applications," *Toxicology Reports* 14 (2025): 101900, https://doi.org/10.1016/j.toxrep.2025.101900.

53. P. Trivedi, G.-S. Liu, L. Klavins, et al., "Scratching the Surface: The Diversity and Roles of Triterpenoids in Plant Cuticular Waxes," *Phytochemistry Reviews* 25 (2025): 2149–2181, https://doi.org/10.1007/s11101-025-10191-w.

Supporting Information

Additional supporting information can be found online in the Supporting Information section.

Supporting File 1: ejlt70120-sup-0001-FigureS1.pptx.

Supporting File 2: ejlt70120-sup-0002-TableS1.docx.

Supporting File 3: ejlt70120-sup-0003-fileS1.pptx.

Supporting File 4: ejlt70120-sup-0004-fileS2.pptx.

Supporting File 5: ejlt70120-sup-0005-fileS3.pptx.

Supporting File 6: ejlt70120-sup-0006-fileS4.pptx.

Supporting File 7: ejlt70120-sup-0007-fileS5.pptx.