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Cutin Composition of Five Finnish Berries

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The raw cutin (i.e., extractive-free isolated cuticular membrane) fraction from Finnish berries, sea buckthorn (*Hippophaë rhamnoides*), black currant (*Ribes nigrum*), cranberry (*Vaccinium oxycoccos*), lingonberry (*Vaccinium vitis-idaea*), and bilberry (*Vaccinium myrtillus*), was depolymerized by NaOMe-catalyzed methanolysis. The composition of cutin monomers was determined by GC-(EI)MS analysis either as methyl esters or as TMSi esters, with OH groups derivatized to TMSi ethers. There was a notable difference in the degree of depolymerization, ranging from 6 to 47%. The extractive-free berry cuticle, that is, raw cutin, thus contains <50% polyester polymer cutin. The predominant cutin monomers were C_{16} and C_{18} ω-hydroxy acids with midchain functionalities, mainly epoxy and hydroxyl groups. Typically, the major compounds were 9,10-epoxy-18-hydroxyoctadecanoic acid, 10,16-dihydroxyhexadecanoic acid, 9,10,18-trihydroxyoctadecanoic acid, 9,10-epoxy-18-hydroxyoctadec-12-enoic acid, and 18-hydroxyoctadec-9-enoic acid. The amount of epoxyacids was rather high in sea buckthorn (∼70%) and cranberry (∼60%), compared with the other berries. The black currant cutin differed from that of the other berries with a significant portion of hydroxyoxohexadecanoic acid (∼12% of total monomers). This investigation of the cuticular hydroxy acids of five Finnish berries is part of the exploitation of the northern natural resources related to the chemical composition, nutritional value, and sensory properties.

KEYWORDS: ***Hippophaë rhamnoides*; sea buckthorn; Eleagnaceae*; Ribes nigrum*; black currant; Grossulariaceae; *Vaccinium myrtillus*; bilberry; Ericaceae; *Vaccinium oxycoccos*; cranberry; *Vaccinium vitis-idaea*; lingonberry; cutin; epoxy fatty acids; hydroxy fatty acids; mass spectrometry**

INTRODUCTION

Cutin is a polyester polymer composed of a complex mixture of interesterified, long-chain ω-hydroxy acids with typically a 16- or 18-carbon skeleton. The fatty acids are commonly substituted with secondary functional groups such as hydroxyl and epoxy groups. Minor proportions of long-chain fatty acids, diacids, alcohols, aromatic compounds, and glycerol increase the extreme diversity of cutin (*1*−*3*). The plant cuticle comprises the insoluble cutin and the nondegradable cutan and soluble cuticular waxes. In addition, trace amounts of polysaccharides, phenols, and amino acids may also be present in the plant cuticle (*3*, *4*). Cutin is considered to be the major constituent of the cuticle, corresponding up to 80% of its dry weight (*3*).

Cuticle is unique for aerial plants. It is attached to the outer epidermal cells via a pectinaceous layer (*1*). Cuticle acts not only as a barrier but also as a gateway between the plant and its environment. Cutin plays an important role in cuticle as a structural component and as a defense barrier toward pathogens and the uncontrolled loss of water, as well as in transporting substances across the plant tissues (*3*, *4*). Its function in protecting the plant against physical, chemical, and biological aggressions have been well characterized (*3*).

Cutin monomers build up a complex, three-dimensional network by cross-links through the primary and secondary hydroxyl and carboxyl groups (*1*, *3*, *4*). It has been shown that the primary hydroxyl groups are almost all esterified, building linear chains. About half of the secondary hydroxyl groups are involved in ester cross-links or branching. Cutin may be depolymerized with ester-breaking reactions or enzymatically with hydrolases, for example, cutinases. The monomers released by chemical methods depend on the reagent used, and enzymatic treatments usually yield oligomeric fragments (*1*, *2*). Once all of the wax and cutin components have been removed from an isolated cuticle preparation, there is usually remaining residual material left. In many species this residue is predominantly polysaccharide, but in some cases it contains also significant amounts of cutan, that is, non-ester cutin (*5*, *6*). It is believed to consist of cutin monomers and polymethylenic chains cross-linked with ether bonds with the presence of double bonds and free carboxyl groups (*1*, *4*, *5*).

Cutin monomers have been widely investigated, but the exact nature of the linkages and the three-dimensional polymeric structures remains unclear. Cutin is held together mainly by ester bonds, although other types of linkages are also probably present in most plants forming non-ester cutin, that is, cutan. The presence of ether bonds formed in reaction between epoxy and hydroxyl groups has also been reported (*1*, *4*). The structure of

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the polymer depends on the chain lengths and on the number and position of esterifiable groups in monomers (*4*). The presence of epoxy acids in monomers affects the degree of cross-linking of the polyester and has influence on the development and the structure of the whole cuticular membrane (*7*). The complexity of the cross-linkages in cutin has been found to increase with aging of the plant (*4*, *6*).

Large variation has been found in cuticular fine structure and in the amount of waxes. The structure of the cuticle and the composition of cutin vary greatly according to the plant species, age, and organ (fruit, leaf, etc.) and according to the stage of growth (*1*, *4*, *6*). In most plants, cutin and cutan may occur in any ratio, differing in their relative amounts at different stages of cuticle development. Some plant cuticles appear to lack cutan completely (e.g., tomato fruit *Lycopersicon esculentum*); others have only cutan (e.g., *Beta vulgaris*), and some may have both (e.g., *Agave americana* leaf) (*3*, *5*, *6*). Variations in the composition of cutin, and the occurrence of different reactive groups such as epoxides, may influence the penetration and interaction of chemicals as well as the protecting capabilities (*4*, *7*). The wide variation in the structure resulting from the chemical diversity may explain the different functions of cuticle.

Dietary fiber has a complex and highly variable composition. It is principally composed of polysaccharides of cell walls, but many edible plants have cell types that contain hydrophobic polymers, for example, cutin, suberin, and lignin. The composition of aliphatic monomers in the cutin of berries has not been widely studied. Only a few references can be found for cranberry (*Vaccinium macrocarbon*) and black currant (*Ribes nigrum*) (*7*, *9*, *10*), but the monomer profile is missing. In addition, some of these studies have been done before development of methods that revealed the epoxy acids as essential parts of cutin monomers (*7*). Our aim was to investigate the cuticular hydroxy acids in five Finnish berries as a part of the exploitation of the northern natural resources related to the chemical composition, nutritional value, and sensory properties. Although the cuticular material contributes only a minor fraction to the whole mass of fruits and berries, it could have an important role as a component of insoluble fiber. The results of animal carcinogenesis studies are variable, but sources of insoluble dietary fibers appear to be more protective than soluble dietary fibers (*8*).

MATERIALS AND METHODS

Berry Material. Lingonberry (*Vaccinium vitis-idaea*) and bilberry (*Vaccinium myrtillus*) samples were powdered whole berries, and black currant (*Ribes nigrum*) and cranberry (*Vaccinium oxycoccos*) were powdered press residues from juice processing from Biokia Oy (Suomussalmi, Finland). Sea buckthorn (*Hippophaë rhamnoides* ssp. *mongolica* var. Trophimovskaya) material investigated was press residues from the whole berries, from which seeds were manually separated and the soft parts dried in an oven (60 °C). Berries were collected at optimal ripeness for industrial berry processing.

Isolation and Exhaustive Extraction of Cuticular Membranes. Cuticular membranes were isolated by overnight enzymatic treatment of the dried berry raw materials with cellulase (5 g/L Econase CE) (AB Enzymes, Darmstadt, Germany) and pectinase (1 g/L Pectinex Ultra SP-L) (Novozymes, Bagswaerd, Denmark) in acetate buffer (5 mM, pH 4). Soluble material was exhaustively extracted in a Soxhlet, first with $CHCl_3$ (12−20 h) and then with MeOH (12−20 h). Extraction times depended on the amount of soluble cuticular waxes in each berry. The procedures were repeated to yield pure extractive-free cuticular membranes, that is, raw cutin, which was washed with water and freeze-dried before depolymerization.

Depolymerization of Raw Cutin. Depolymerization of the isolated raw cutin was carried out by as described previously (*7*) with small modifications. Dried samples of extractive-free raw cutin (50−100 mg) were refluxed for 3 h in 25 mL of a 1.3 or 2 M solution of NaOMe in MeOH. The reagent used was freshly prepared by dissolving metallic sodium in dry methanol. Precautions were taken to exclude moisture from the reaction. The reaction mixtures were filtered, and residues were refluxed for an additional 15 min with 25 mL of MeOH. The combined alcoholic filtrates were acidified by the addition of 2 M H_2SO_4 in MeOH and taken to dryness using a rotary evaporator. The residue was suspended in 50 mL of H_2O and extracted with $CHCl_3$ (2 × 50 mL). The organic phase containing the monomers was dried over anhydrous Na_2SO_4 and evaporated to dryness. Cutin monomers were determined gravimetrically and stored in $CHCl_3$ in a freezer for further analysis.

Compositional Analysis and Identification of Compounds. Cutin monomers, partially as methyl esters, were derivatized before the chromatographic analysis. $CHCl_3$ was evaporated, and the residue was dried under a stream of nitrogen and kept in a desiccator overnight before trimethylsilylation with TriSil reagent (HMDS and TMCS in pyridine) (Pierce Chemicals Co., Rockford, IL) at 60 °C for 15 min.

The monomer composition was determined by GC-EIMS with a Shimadzu GC MS-QP5000 (Shimadzu, Kyoto, Japan) under the following chromatographic conditions: 30 m × 0.25 mm × 0.25 μm EC-1 column (Alltech Associates Inc., Deerfield, IL); split injection (14:1); carrier gas, He (linear velocity 42 cm/s); injector and detector temperatures, 300 °C. The oven temperature was programmed from 125 °C at 10 °C/min to 205 °C, from 205 °C at 5 °C/min to 290 °C, and kept for 10 min. EI-MS spectra was obtained at 70 eV ionization energy. **Figure 1** shows examples of the total ion chromatogram obtained from derivatized sea buckthorn, black currant, and bilberry methanolysates.

Components were identified by comparison of their EI-MS spectra of their TMSi derivatives (as methyl ester TMSi ether or as TMSi ester TMSi ether) with published spectra (*7*, *10*−*14*) and typical fragmentation patterns with the aid of retention times and indices. Retention indices for the components were determined with a hydrocarbon standard (DRH-008S-R2) (Accustandard, New Haven, CT) and calculated with the Kovats index. The positions of the double bonds were not confirmed by chemical methods.

RESULTS AND DISCUSSION

Cutin Depolymerization Yields. Raw cutin (i.e., extractive-free isolated cuticular membrane) from five berry varieties was depolymerized by NaOMe-catalyzed methanolysis. Monomer yield was determined gravimetrically as the $CHCl_3$-soluble material after methanolysis. Two different NaOMe concentrations were tested (1.3 and 2 M), but as the concentration had little or no effect on the cutin monomer yield (data not shown), results from both concentrations were combined (**Table 1**). Yields of obtained depolymerized material varied widely between different berries, ranging from 6% in bilberry (*Vaccinium myrtillus*) to 46% in sea buckthorn (*Hippophaë rhamnoides*).

Usually the cutin depolymerization yields have been >50% of the raw cutin in plants. Cutin is known to represent ∼50−60% of the weight of the isolated apple fruit membranes obtained with transesterification (*15*). We have obtained from apple peel yields of up to 80% (unpublished results). Over 70% of membrane material from tomato peels is released as cutin monomers, the remaining part being mostly carbohydrates (*16*). The typical yield in alkaline hydrolysis of lime fruit cutin is 60−65% and in transesterification ∼50% (*17*). This resistance to hydrolytic breakdown of lime fruit raw cutin may be attributed to non-ester cross-links within the polymeric structure. The unreacted residue has been found to consist almost exclusively of rigid, solid-like functional groupings, determined with solid-state ^{13}C NMR (*17*). This could be the case for berry cutins as well, but the residual material may also be a mixture of polymers, for example, polysaccharide and non-ester cutin, or

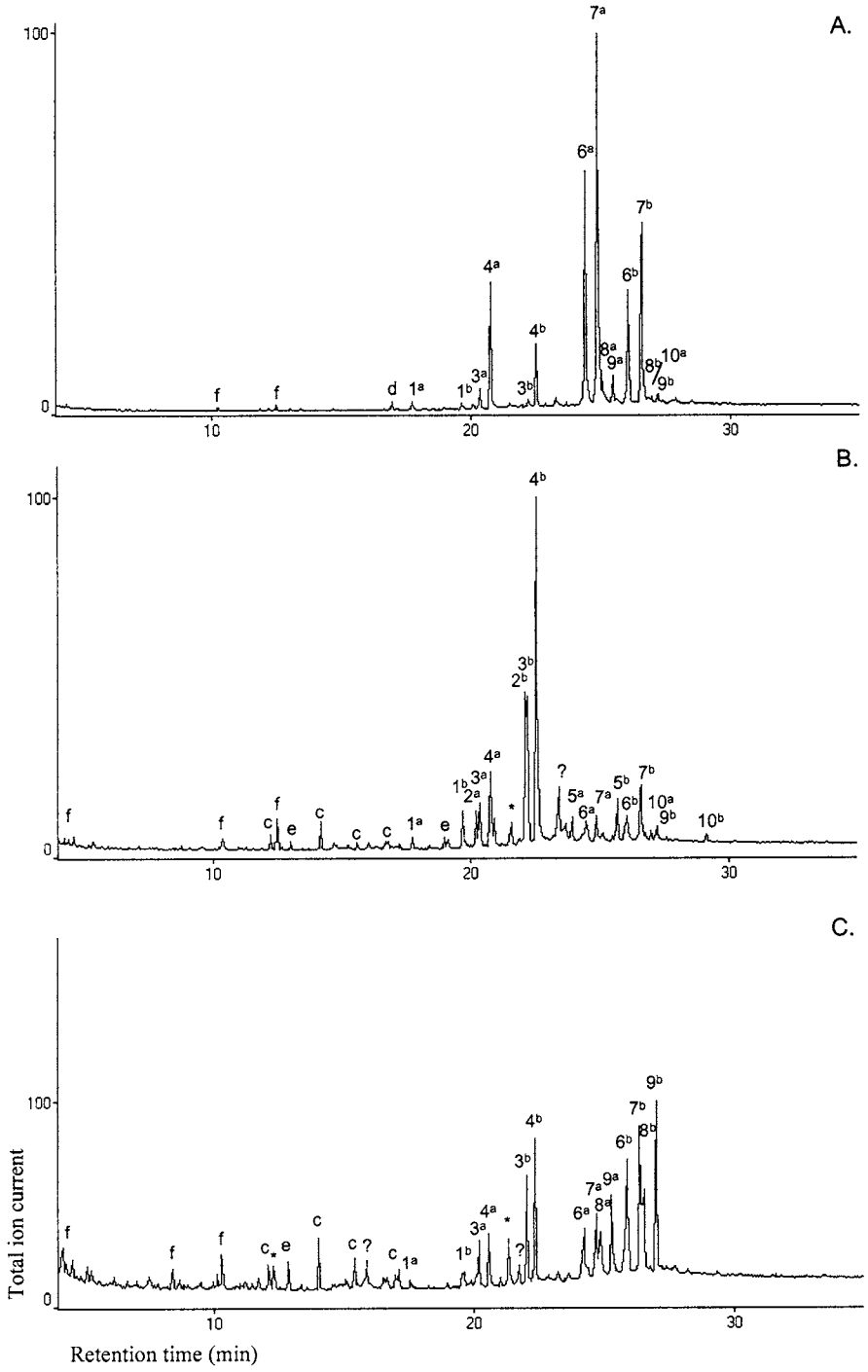

Figure 1. Total ion chromatogram obtained from derivatized sea buckthorn (**A**), black currant (**B**), and bilberry (**C**) methanolysates.

Table 1. Cutin Monomer Yields as Percentage of the Extractive-free Raw Cutin by Methanolysis

sample	cutin monomer yield[a] (%)
sea buckthorn (*Hippophaë rhamnoides*)	46
black currant (*Ribes nigrum*)	8
cranberry (*Vaccinium oxycoccos*)	27
lingonberry (*Vaccinium vitis-idaea*)	30
bilberry (*Vaccinium myrtillus*)	6

[a] Determined gravimetrically of $CHCl_3$-soluble material from NaOMe methanolysis, SD = 1–3%.

cutan. Because the composition of this residual material is not well understood and structural studies have not been carried out in our experiments, we have not been able to make any conclusions about the amount and composition of cutan in the berry cuticles. Apparently, berry cuticle is quite resistant to ester-breaking reactions, which may be due to the high amount of cutan present. Cuticles from some berries seem to almost totally lack the ester-linked cutin. This may have a strong influence on the various functions of cuticle in berries and also as dietary fiber.

Cutin Monomer Compositions. The compositions of the hydroxy acid monomers released from the raw cutin fraction of the berries by alkaline transesterification were identified by GC retention times and EI mass spectra. The analyses were carried out as TMSi ethers of methyl or TMSi esters. Only a few reference compounds were available. The retention behavior

Table 2. Important Ions in Mass Spectra of Cutin Monomers and Calculated Kovats Indices

	important ions in mass spectra (*m*/*z*)						
ω-hydroxy acid[a]	methyl ester TMSi ether[b]	ID[c]	I_k[d]	TMSi ester TMSi ether[b]	ID[c]	I_k[d]	refs
16-hydroxyhexadecanoic	343 [M – 15]$^+$, 327 [M – 31]$^+$, 311 [M – 47]$^+$, 59 and 146,* 103, 89, 75, 73, 55	S, R	2268	401 [M – 15]$^+$, 385 [M – 31]$^+$, 311 [M – 105]$^+$, 217 and 204,* 147, 117, 103, 75, 73, 55	S, D	2390	*11*, *12*
16-hydroxy-10-oxohexadecanoic (main isomer)	357 [M – 15]$^+$, 341 [M – 31]$^+$, 325 [M – 47]$^+$, 214, 126, 111, 83, 75, 73, 55	D	2425	415 [M – 15]$^+$, 272, 251, 215, 201, 126, 111, 83, 75, 73, 55	D	2548	*10*
18-hydroxyoctadec-9-enoic	384 [M]$^+$, 369 [M – 15]$^+$, 353 [M – 31]$^+$, 337 [M – 47]$^+$, 159 and 146,* 129, 109, 95, 75, 73, 55	R, D	2435	442 [M]$^+$, 427 [M – 15]$^+$, 411 [M – 31]$^+$, 383, 337 [M – 105]$^+$, 217 and 204,* 147, 135, 129, 117, 109, 95, 75, 73, 55	D	2555	*11*
dihydroxyhexadecanoic (major 10,16-diOH, minors 9,16 and 8,16-diOH)	431 [M – 15]$^+$, 415 [M – 31]$^+$, 399 [M – 47]$^+$, 317, 303, 289, 275, 273, 259, 245, 159 and 146,* 147, 129, 103, 95, 75, 73, 55	R, D	2460	489 [M – 15]$^+$, 399 [M – 90 – 15]$^+$, 383 [M – 105]$^+$, 331, 317, 303, 289, 275, 217 and 204,* 147, 129, 103, 95, 75, 73, 55	D	2575	*11*, *13*
20-hydroxyeicosanoic	399 [M – 15]$^+$, 383 [M – 31]$^+$, 367 [M – 47]$^+$, 159 and 146,* 103, 89, 75, 73, 55	D	2665	457 [M – 15]$^+$, 441 [M – 31]$^+$, 367 [M – 105]$^+$, 217 and 204,* 147, 129, 117, 103, 75, 73, 55	D	2783	*14*
9,10-epoxy-18-hydroxyoctadec-12-enoic	471 [M – 31]$^+$, 383, 303, 271, 259, 243, 213, 191, 155, 129, 121, 109, 103, 89, 75, 73, 55	R, D	2700	545 [M – 15]$^+$, 441, 423, 361, 329, 317, 301, 271, 259, 191, 149, 129, 121, 109, 103. 75, 73, 67	D	2808	*7*
9,10-epoxy-18-hydroxyoctadecanoic	489 [M – 15]$^+$, 473 [M – 31]$^+$, 457 [M – 47]$^+$, 385, 303, 274,* 259, 201, 155, 129, 121, 109, 103, 81, 75, 73, 55	R, D	2731	547 [M – 15]$^+$, 443, 425, 332, 317, 303, 243, 147, 129, 121, 109, 103, 75, 73, 67	D	2840	*7*
9,10,18-trihydroxyoctadec-12-enoic	545 [M – 15]$^+$, 361, 317, 271, 259, 155, 147, 129, 109, 103, 75, 73	R, D	2743	513 [M – 105]$^+$, 419, 329, 317, 301, 217 and 204,* 191, 147, 129, 109, 103, 75, 73	D	2848	*12*
9,10,18-trihydroxyoctadecanoic	547 [M – 15]$^+$, 332,* 303, 259, 243, 212, 155, 147, 129, 109, 103, 81, 75, 73	R, D	2771	515 [M – 105]$^+$, 390, 317, 303, 217 and 204,* 147, 129, 109, 103, 81, 75, 73	D	2878	*11*, *12*
22-hydroxydocosanoic	442 [M]$^+$, 427 [M – 15]$^+$, 411 [M – 31]$^+$, 395 [M – 47]$^+$, 159 and 146,* 129, 103, 83, 75, 73, 55	R, D	2863	485 [M – 15]$^+$, 469 [M – 31]$^+$, 395 [M – 105]$^+$, 217 and 204,* 147, 129, 117, 103, 75, 73, 55	R, D		*14*

[a] Analyzed as methyl ester TMSi ethers and TMSi ester TMSi ethers. [b] Asterisks indicate typical rearrangement ions. [c] S, identified by comparison to standard compound; R, identified by comparison to published spectrum; D, deduced according to the mass spectrum in the present study. [d] Kovats index.

Table 3. Approximate Composition (Percent) of Cutin Monomers Released from Five Berries by Methanolysis

peak[a]	ω-hydroxy acid[b]	sea buckthorn	lingonberry	cranberry	bilberry	black currant
1	16-hydroxyhexadecanoic	1	1	1	1	3
2	16-hydroxy-10-oxohexadecanoic (main isomer)					12
3	18-hydroxyoctadec-9-enoic[d]	2	6	6	9	13
4	dihydroxyhexadecanoic (major, 10,16-diOH; minor, 9,16 and 8,16-diOH)	15	20	9	13	30
5	20-hydroxyeicosanoic	1			tr[e]	4
6	9,10-epoxy-18-hydroxyoctadec-12-enoic[c,d]	30	11	4	11	3
7	9,10-epoxy-18-hydroxyoctadecanoic[d]	41	28	56	15	8
8	9,10,18-trihydroxyoctadec-12-enoic[d]	2	6	1	8	1
9	9,10,18-trihydroxyoctadecanoic	3	23	20	18	2
10	22-hydroxydocosanoic	tr	tr	1	1	1
	total hydroxy acids	94	95	97	77	76
	Other Compound Groups[b]					
aromatic compounds (comprises also hydroxy acids)		1	1	tr	6	4
long-chain aliphatic acids, diacids, and alcohols		1	1	tr	9	9
unidentified		5	3	3	9	11
total		100	100	100	100	100

[a] Peak number in **Figure 1**. [b] Compounds determined as methyl ester TMSi ether and/or TMSi ether TMSi ester. [c] Determined as corresponding methoxyhydrin compounds. [d] Position of double bond not confirmed by chemical methods. [e] tr, <0.5%, nontraceable.

and the mass spectometric fragmentation patterns were thus the methods of identification (**Table 2**). The cutin monomer mixtures were mostly composed of long-chain aliphatic ω-hydroxy acids (**Table 3**), which are known to commonly exist in cutin polymers of plant materials. **Figure 1** shows examples of total ion chromatograms of cutin monomer mixtures from methanolysis of sea buckthorn, black currant, and bilberry.

C_{16} and C_{18} ω-hydroxy acids, with midchain functionalities such as epoxy and hydroxyl groups, were predominant in all cutins. Also, longer chain ω-hydroxy acids (C_{20} and C_{22}) were found in some cutins as minor monomers (<6%). The other long-chain aliphatic monomers contained alkanoic acids, ω-dialkanoic acids, and alkanols. These were found mainly in black currant and bilberry, comprising <10% of the total monomers identified. Aromatic compounds were also found as minor components (<5%) of the cutins studied. Benzoic acid, <1%, and coumaric acid isomers were present in most cutins, coumaric acid isomers being the most abundant in black currant (3−4%). In bilberry hydroxymethoxybenzoic and hydroxydimetoxybenzoic acids were also found (1.1 and 2.1%, respectively). The

presence of aromatic acids esterified to the polyesters is an important feature in the cutin polymer and might play a protective role when released by the hydrolytic enzymes excreted by fungi (*18*).

Saturated acids predominated over unsaturated acids in each of the berries studied. In sea buckthorn and bilberry the proportion of unsaturated components was higher than in other berries but still, in both cases, <35% of total acids. In all other cutins C_{18} monomers were dominant except in black currant, in which the main monomers were various dihydroxyhexadecanoic acid isomers. The ratio of C_{16} to C_{18} compounds ranged from ca. 2:1 in black currant to ca. 1:5 in bilberry and ca. 1:9 in cranberry. The cutin monomer composition of black currant differed from the other berry cutins. 16-Hydroxy-10-oxohexadecanoic acid and related positional isomers comprised ~12% of total monomers in black currant cutin, which is in accordance with previously reported (~14%) information (*10*). This monomer was absent in all other berry cutins.

Dihydroxyhexadecanoic acids are usually important constituents of most plant cutins. In this study, several isomers were also found in berry cutins. Dihydroxyhexadecanoic isomers have been found in many cutin hydrolysates, the major isomers being 10,16- and 9,16-dihydroxyhexadecanoic acids with isomers 8,16 and 7,16 occurring in smaller quantities (*13*). Of the berry cutins investigated only in cranberry was the amount of dihydroxyhexadecanoic acids <10% of total monomers. In black currant these compounds were the main monomers, as previously mentioned (*7*). The predominant isomer was 10,16-dihydroxy acid, with smaller amounts of the 9,16 and 8,16 isomers. These compounds elute as a single peak from the EC-1 column used, but the structures can be recognized from the mass spectra of the derivatives, because the main fragments are caused by α-cleavage relative to the functional group. The presence of dihydroxyhexadecanoic acids in the depolymerisates is thought to be a useful single indicator for cutin, which differentiates it from other polymers in plants, for example, suberin (*19*).

The epoxy compounds were converted into corresponding methoxyhydrin compounds during depolymerization of cutin by methanolysis. The amount of epoxy acids varied from ~70% in sea buckthorn to ~10% in black currant. It has been previously reported that epoxy acids may comprise up to 60% of the total monomeric units of cutin polymers (*7*). Polymers containing high amounts of epoxide groups are essentially linear polyesters because epoxy groups cannot be involved in esterification. Thus, the polymer structure made up mostly of epoxy monomers differs greatly from di- or trihydroxy monomer-based polymers. Epoxy polymers may resemble those consisting mainly of ω-hydroxy acids or α,ω-diacids.

As a curiosity, it is worth mentioning that several hydroxy and epoxy fatty acids have been reported to exist in the seed oil of sea buckthorn. The major epoxy acid was claimed to be 15,16-epoxy-9,12-octadecadienoic acid (*20*). Thirteen monohydroxy and four dihydroxy acids were also identified (*21*). It has to be taken into account that some of the compounds identified might have been derived from unsaturated fatty acids or their derivatives via autoxidation or even via hydrolytic cleavage from polymeric material.

The monomer profile of cranberry has been previously investigated (*9*), but the method used is not compatible with current cutin definition (ester-linked polymer). Cutin was hydrolyzed with methanolic KOH followed by cleaving of the peroxide and ether linkages with NaI and HI, respectively. The monomer composition was thus possibly a mixture of cutin and cutan monomers. The method also fails in separating epoxyhydroxy acids from other monomers.

Even though precautions were taken to eliminate moisture from the reaction, there were also free acids among the methyl esters in all of the end products. Thus, the monomers were determined both as methyl ester TMSi ethers and as TMSi ester TMSi ethers. Response factors for both derivative types were comparable, on the basis of comparison of methyl ester TMSi ether and TMSi ester TMSi ether of 12-hydroxyoctadecanoic acid. The amounts of the components were the same in different analyses with one sample material, even when the amounts of the derivatives (ratio of methyl ester TMSi ethers and TMSi ester TMSi ethers) were different. According to newer methods of analysis (e.g., NMR), it is possible that free acids are present among the esterified monomers (*22*). However, in our results the variation in the amount of free acids indicates some kind of saponification/acidification during or after the methanolysis. The injected sample concentrations were comparable, but the intensities in total ion chromatograms (TIC) varied clearly according to the berry species. This may be due to some of the compounds released by depolymerization not being volatile enough to be analyzed under the GC conditions used in this study. Glycerol is known to be a relevant monomer (1−14% of total monomers) of some cutins (*2*), but in this study glycerol was not determined due to the method used to recover monomers after depolymerization.

This study was part of the exploitation of the northern natural resources related to the chemical composition, nutritional value, and sensory properties. Information on the insoluble polymers in berries is important for understanding, for example, their dietary properties. There is little information on the cutin monomer composition of edible plants. The most important finding in this study was related to the depolymerization yield of isolated raw cutin, which varied widely, between 6% in bilberry and 46% in sea buckthorn.

ABBREVIATIONS USED

HMDS, hexamethyldisilizane; TMCS, trimethylchlorsilane; GC-EIMS, gas chromatography−electron impact mass spectrometry; TMSi, trimethylsilyl.

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