Berry cuticular wax: Composition, morphology, biosynthesis and effect of environmental factors

Priyanka Trivedi[1], Katja Karppinen[1], Nga Nguyen[1], Petri Sundqvist[2], Juha Väänänen[2], Janne Remes[2], Esa Heinonen[2]
Maris Klavins[3], Laura Jaakola[4,5], Hely Häggman[1]

[1]Department of Ecology and Genetics, University of Oulu, Finland
[2]Center of Microscopy and Nanotechnology, University of Oulu, Finland
[3]Department of Environmental Science, University of Latvia, Latvia
*[4]Norwegian Institute of Bioeconomy Research, NIBIO, Å*s*, Norway, [5]Climate laboratory Holt, Department of Arctic and Marine Biology, UiT The Arctic University of Norway, Tromsø, Norway*
priyanka.priyanka@oulu.fi

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Introduction

The natural cuticular wax of berry is either embedded (intracuticular wax) or deposited on the surface (epicuticular wax) as a protective layer called cuticle. Cuticle acts as an interface between the plant and the environment. The wax layer, being outermost prevents non stomatal water loss and mediates interaction with various biotic and abiotic factors.

Due to specific chemical composition and morphology of berry wax it also has potential industrial applications e.g, in the fields of cosmetics and medicine. Berry waxes are derieved from fatty acids modified to form very long chain hydrocarbons, alcohols, aldehydes, ketones and wax esters of fatty acids and alcohols. The epicuticular waxes form complex three dimensional structures resulting in various morphologies.

The aim of this work is to study waxes in finnish wild berries such as Bilberry (*Vaccinium myrtillus* L.), Lingonberry (*Vaccinium vitis-idaea* L.) and Crowberry (*Empetrum nigrum* L.).

Wax Biosynthesis

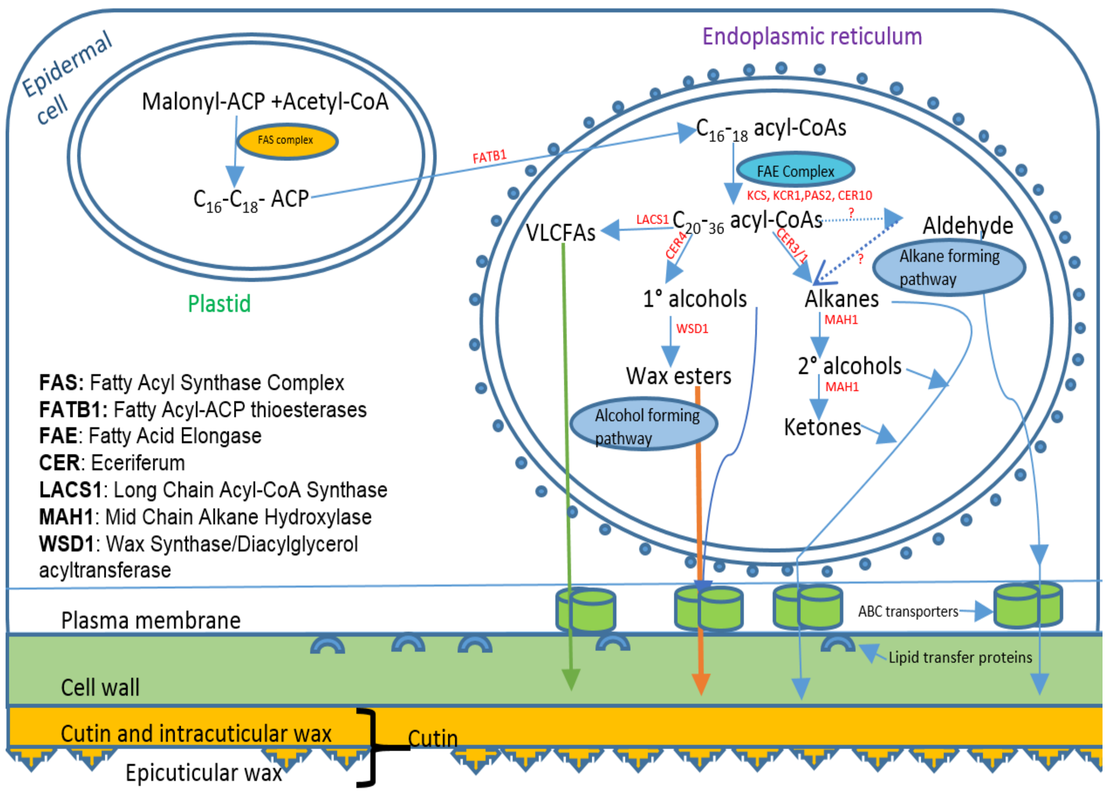


Fig 1: Wax biosynthetic pathway (Samuels et al., 2003; Joubès et al., 2013)

Results

Supercritical Fluid Extraction

The yield for lingonberry fruit wax was 1.02% (10.2 mg wax/g dry weight). For bilberry fruit, the yield was found to be 0.45% (4.51 mg wax/g dry weight).

Extracted lingonberry fruit wax

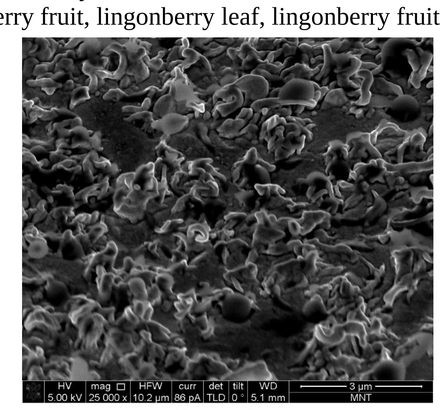
Extracted bilberry fruit wax

Wax Compositional Analysis

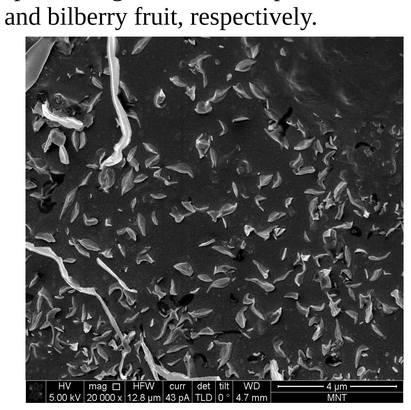
The wax of lingonberry and bilberry fruit was composed of triterpenoids, alkanes, fatty acids, alcohols and aldehyde. Health beneficial compounds such as α-tocopherol, phytosterols (β-sitosterol, lanosterol, taraxasterol) and triterpeniods (β-amyrin, α-amyrin, lupeol) were also identified.

Wax Surface Morphology

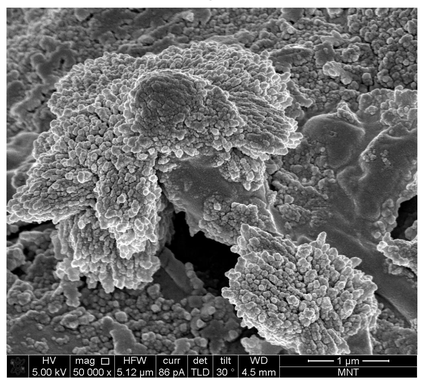
Different wax surface morphologies have been found in plants (Barthlott et al. 1998). We observed distinct wax crystalloid forms such as membranous platelets, granules, entire platelets and rosettes in crowberry fruit, lingonberry leaf, lingonberry fruit and bilberry fruit, respectively.



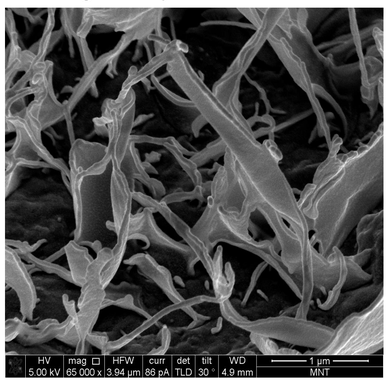
Bilberry fruit



Lingonberry fruit



Lingonberry leaf



Crowberry fruit

Methods

Plant material

Wax was extracted from pressed cakes of bilberry and lingonberry obtained from leftovers of the juice industry. Cakes were dried in oven at 60°C and milled to powder of dimensions less than 1mm.

Frozen lingonberry fruit cake

Dried and milled lingonberry fruit cake loaded into a supercritical CO_2 extraction vessel

SFE (Supercritical Fluid Extraction)

Supercritical Fluid Extraction

Supercritical fluid extraction was done at a pressure of 350 bars, temperature 60°C with a CO_2 flow rate of 0.4 to 0.5 liters per minute. 10 litre extractions were done for bilberry and lingonberry.

Compositional Analysis

Compositional analysis of extracted wax was done using GC-MS. The fatty acid composition of wax was determined by gas chromatography of fatty acid methyl esters (FAME) as described Dobson et al., 2012.

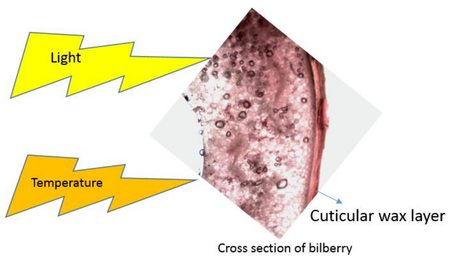
Scanning Electron Microscopy

The freeze dried berry samples were analyzed for surface micromorphology using FEI Helios dual beam scanning microscope.

Future work

Effect of environmental factors on wax biosynthesis

Plants are subjected to various types of abiotic stresses. The wax layer protects plants from harmful effects of light, temperature, osmotic stress and physical damage. We will study the effect of temperature and light on wax biosynthesis, composition and morphology. In bilberry, we will also study gene expression changes related to wax biosynthesis during berry development. Bilberry transcriptome data has recently been created by our research group and it will be utilized in search for genes related to wax biosynthesis



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References

1. *Dobson G, Shrestha M, Hilz H, Karjalainen R, McDougall G, Stewart D, Lipophilic components in black currant seed and pomace extracts, Eur. J. Lipid Sci. Technol. 2012, 114, 575–582.*
2. *Barthlott W, Ditsch CF, Meusel I and Wilhelmi H, Classification and terminology of plant epicuticular waxes, Bot. J. Linn. Soc. 1998, 126, 237-260*
3. *Kunst L, Samuels AL, Biosynthesis and secretion of plant cuticular wax, Prog. Lipid Res. 2003, 42, 51–80*
4. *Bernard A, Joubès J, Arabidopsis cuticular waxes: Advances in synthesis, export and regulation, Prog. Lipid Res. 2013, 52, 110–129*