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TURKU, FINLAND – 2023

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ISBN 978-951-29-9568-4 (print)
ISBN 978-951-29-9569-1 (pdf)
ISSN 2323-9395 (print)
ISSN 2323-9409 (pdf)
Painosalama Oy – Turku, Finland 2023

In memory of my father

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ABSTRACT

Potato is the most important non-cereal crop worldwide. It has been consumed in the South American Andes for thousands of years and then introduced to Europe in the XVII century. Thanks to its nutritional value and high yield it was then expanded to the rest of the world. Since potato is a staple food crop for many countries, it plays a crucial role in maintaining food security and reducing malnutrition. Unfortunately, the sustainable production of this crop is threatened by climate change that causes rising global temperatures and altering weather conditions all over the world.

The main purpose of this study was to understand how weather variables affected by latitude and year impact the biosynthesis of phenolic compounds and ascorbic acid in pigmented potato cultivars. The first aim of this work was to develop reliable extraction methods for anthocyanins and ascorbic acid, as previous studies showed contradictory information on the anthocyanin content when using fresh, freeze-dried or steamed potatoes. The second aim was to assess the effect of temperature, cultivar, and tuber developmental stage on the expression of genes involved in the biosynthesis of phenolic compounds and the content of anthocyanins, phenolic acids, and ascorbic acid in potato cultivars with purple or yellow flesh and/or skin. The third aim was to evaluate the effect of weather conditions (affected by latitude and year), cultivar, and tuber developmental stage also on the gene expression levels and on the content of anthocyanins, phenolic acids, and ascorbic acid in potato cultivars grown at two different latitudes and two consecutive years in Finland.

The results showed that anthocyanins were most effectively extracted from freeze-dried potatoes using 70 % acidified methanol. Tris(2-carboxyethyl) phosphine hydrochloride was the most effective in reducing dehydroascorbic acid to ascorbic acid and was therefore the most suitable reducing agent for the determination of total ascorbic acid.

Cultivar was the main variable that affected the gene expression levels of the enzymes involved in the biosynthesis of phenolic compounds as well as the anthocyanin and phenolic acid profile. The main anthocyanin in the purple-fleshed and purple-skin cultivars was a petunidin derivative or malvidin derivate (depending on the cultivars), while the main anthocyanin in the red-skinned cultivar was a pelargonidin derivative.

The effect of tuber developmental stage was evaluated in the second and third aim of this study. In both cases, it was found that the content of the main anthocyanins and phenolic acids as well as ascorbic acid tended to decrease with tuber maturation, although the content of minor anthocyanins and phenolic acids did not show a clear tendency to increase or decrease. The gene expression levels also remained relatively stable for most of the genes studied except for F3′H which showed a clear decreasing trend during tuber development in the purple-fleshed cultivars.

When potato tubers were cultivated under controlled temperatures (at 13 and 18 °C), it was found that the studied temperatures did not affect the content of anthocyanins, but mildly affected the content of phenolic acids and ascorbic acid during tuber development. However, when tubers reached full maturity no significant differences in the content of anthocyanins, phenolic acids or ascorbic acid were observed between the tubers grown at the two studied temperatures.

Our third aim was to evaluate the impact of latitude on the content of phenolic compounds and ascorbic acid in potato tubers, and for that we evaluated two latitudes and replicated the experiment in two consecutive years for repeatability purposes. However, when we processed our data, we realized that the synthesis of the metabolites of interest were mainly affected by the interaction of "Latitude: Year" and less by the solo effect of "Latitude" or "Year". So, when potato tubers were cultivated at different latitudes (61.2 and 64.8° N) and years (2014 and 2015) in Finland, potato plants cultivated at 61.2° N in 2014 (Kokemäki) were exposed to temperatures above 18 °C and reduced precipitation events. The pigmented potato tubers produced in this trial had the lowest expression levels of most of the genes involved in the phenylpropanoid pathway and the lowest content of phenolic compounds and ascorbic acid. On the other hand, when potato plants were cultivated at the same latitude (61.2° N) but in 2015 (Köyliö), the plants were exposed to temperatures between 13 and 18 °C and good precipitation events. These plants produced tubers with the highest gene expression levels, as well as the highest contents of anthocyanins and phenolic acids. The potato plants cultivated at 64.8° N in 2014 and 2015 (Muhos) produced tubers with intermediate contents of anthocyanins, phenolic acids, and ascorbic acid. Positive gene-gene, gene-anthocyanins and anthocyanin-anthocyanin correlations were also stronger and more significant at 61.2° N in 2015 (Köyliö) than in the other field trials.

In this study, we observed that a mild increment in temperature combined with poor precipitation events at 61.2° N in 2015 (Kokemäki) had a detrimental effect on the synthesis of bioactive compounds in potato tubers. However, during the last five years, temperatures in Finland have exceeded those of 2015 and Finnish potato growers have reported difficulties due to unexpected weather events. Further research is needed to better understand the potentially synergistic interactions of the main weather variables affecting the synthesis of bioactive compounds in potato tubers.

SUOMENKIELINEN ABSTRAKTI

Viljojen jälkeen peruna on maailman tärkein viljakasvi. Sitä on syöty ensin Etelä-Amerikan Andeilla tuhansien vuosien ajan, josta se tuotiin Eurooppaan XVII vuosisadalla. Ravintoarvonsa ja suuren satonsa ansiosta se levisi sitten muualle maailmaan. Koska peruna on monien maiden perusviljelykasvi, sillä on ratkaiseva merkitys elintarviketurvan ylläpitämisessä ja aliravitsemuksen vähentämisessä. Valitettavasti tämän viljelykasvin kestävää tuotantoa uhkaa ilmastonmuutos, joka nostaa maapallon lämpötiloja ja muuttaa sääolosuhteita kaikkialla maailmassa.

Tämän tutkimuksen päätarkoituksena oli ymmärtää, miten leveyspiirin ja vuodenajan säämuuttujat vaikuttavat fenolisten yhdisteiden ja askorbiinihapon synteesiin pigmentoituneissa perunalajikkeissa. Työn ensimmäisenä tavoitteena oli luotettavan uuttomenetelmän kehittäminen antosyaaneille ja askorbiinihapolle. Aiemmissa tutkimuksissa oli antosyaanipitoisuuksista saatu ristiriitaisia tietoja, tutkittaessa tuoreita, kylmäkuivattuja tai höyrytettyjä perunoita. Toisena tavoitteena oli arvioida lämpötilan, lajikkeen ja mukulan kehitysvaiheen vaikutusta fenolisten yhdisteiden biosynteesiin osallistuvien geenien ilmentymiseen sekä antosyaanien, fenolihappojen ja askorbiinihapon pitoisuuksiin perunalajikkeissa, joiden malto ja/tai kuori on violetti tai keltainen. Kolmantena tavoitteena oli arvioida sääolosuhteiden (joihin vaikuttavat leveysaste ja vuosi), lajikkeen ja mukulan kehitysvaiheen vaikutusta antosyaanien, fenolihappojen ja askorbiinihapon pitoisuuksiin sekä näiden yhdisteiden biosynteesiin osallistuvien geenien ilmentymistasoihin perunalajikkeissa, joita kasvatettiin kahdella eri leveysasteella ja kahtena peräkkäisenä vuonna Suomessa.

Tulokset osoittivat, että antosyaaneja saatiin eniten uuttamalla kylmäkuivattuja perunoita happamoidulla 70 % metanolilla. Tris(2-karboksietyyli) fosfiinihydrokloridi oli tehokkain pelkistämään dehydroaskorbiinihappo askorbiinihapoksi ja siten soveltui parhaiten askorbiinihapon kokonaismäärän määrittämiseen.

Perunalajike oli tärkein muuttuja, joka vaikutti geeniekspressioprofiiliin sekä antosyaani- ja fenolihappoprofiiliin. Pääantosyaani violettimaltoisissa ja -kuorisissa lajikkeissa oli petunidiinijohdannainen tai malvidiinijohdannainen (lajikkeesta riippuen), kun taas punakuorisen lajikkeen pääantosyaani oli pelargonidiinijohdannainen.

Mukulan kehitysvaiheen vaikutusta arvioitiin tämän tutkimuksen toisessa ja kolmannessa tavoitteessa, ja molemmissa tapauksissa havaittiin, että tärkeimpien antosyaanien ja fenolihappojen sekä askorbiinihapon pitoisuudet vähenivät yleensä kypsymisen myötä. Fenolihappojen pitoisuuksissa ei havaittu systemaattisia eroja kasvukauden aikana. Myös geenien ilmentymistasot pysyivät suhteellisen vakaina useimpien tutkittujen geenien osalta lukuun ottamatta

F3′H:ta, jonka ilmeneminen selvästi väheni violettimaltoisissa lajikkeissa mukulan kypsyessä.

Kolmantena tavoitteena oli arvioida leveysasteen vaikutusta perunan mukuloiden fenolisten yhdisteiden ja askorbiinihapon pitoisuuksiin. Perunoita kasvatettiin kahtena eri vuonna kahdella eri leveysasteella. Tulokset osoittivat, että tutkittavien parametrien "leveysaste" ja "vuosi" erillisvaikutukset aineenvaihduntatuotteiden synteesiin olivat vähemmän merkittäviä kuin ko. parametrien yhdysvaikutus "leveysaste: vuosi". Kun perunamukuloita viljeltiin eri leveysasteilla (61,2 ja 64,8° N) ja eri vuosina (2014 ja 2015), havaittiin, että perunakasvit, jotka kasvoivat kokeessa leveysasteella 61,2° N vuonna 2014 (Kokemäki), altistuivat yli 18 °C:n lämpötiloille ja vähäisemmille sateille. Tällöin kehittyi perunamukuloita, joissa fenyylipropanoidireittiin osallistuvien geenien ilmentymistasot olivat alentuneet ja antosyaanien, fenolihappojen ja askorbiinihapon pitoisuudet olivat vähentyneet. Toisaalta perunakasvit, jotka kasvoivat kokeessa leveysasteella 61,2° N vuonna 2015 (Köyliö), altistuivat runsaille sateille ja 13-18 °C:n lämpötiloille, ja ne tuottivat mukuloita, joiden geenien ilmentymistasot sekä antosyaanien ja fenolihappojen pitoisuudet olivat korkeimmat. Vuosina 2014 ja 2015 Muhoksella (64,8° N) kasvatetut perunat tuottivat mukuloita, joiden antosyaani-, fenolihappo- ja askorbiinihappopitoisuudet olivat keskitasoa. Positiiviset geeni-geeni-, geeni-antosyaani- ja antosyaani-antosyaani-korrelaatiot olivat myös vahvempia ja merkitsevämpiä kuin muissa kenttäkokeissa.

Tässä tutkimuksessa havaitsimme, että vuonna 2015 lämpötilan lievä nousu yhdistettynä vähäisiin sademääriin leveysasteella 61,2° N (Kokemäki) vaikutti haitallisesti perunan mukuloiden bioaktiivisten yhdisteiden synteesiin. Viimeisten viiden vuoden aikana Suomen lämpötilat ovat kuitenkin ylittäneet vuoden 2015 lämpötilat, ja suomalaisten perunanviljelijöiden on raportoitu kohdanneen vaikeuksia odottamattomien sääilmiöiden vuoksi. Lisätutkimusta tarvitaan, jotta voidaan ymmärtää paremmin perunan mukuloiden bioaktiivisten yhdisteiden synteesiin vaikuttavien tärkeimpien säämuuttujien mahdollisesti synergistiset yhteisvaikutukset.

LIST OF ABBREVIATIONS

3ORT	UDP-rhamnose anthocyanidin-3-glucoside rhamnosyltransferase
4CL	4-coumarate-CoA ligase
AA	Ascorbic acid
ANOVA	Analysis of variance
ANS	Anthocyanin synthase
AOMT3	Flavonoid 3′,5′-methyltransferase-like
APX	Ascorbate peroxidase
ASCA	ANOVA-simultaneous component analysis
C3H	4-coumarate 3-hydroxylase
C3′H	*p*-coumaroyl shikimate 3′-hydroxylase
C4H	Cinnamate 4-hydroxylase
CHI	Chalcone isomerase
CHS	Chalcone synthase
CSE	Caffeoyl shikimate esterase
DAD	Diode array detector
DAP	Days after planting
DFR	Dihydroflavonol 4-reductase
DHA	Dehydroascorbic acid
DHAR	Dehydroascorbate reductase
DTT	Dithiothreitol
EDTA	Ethylenediaminetetraacetic acid
ESI-MS	Electrospray ionisation - mass spectrometry
F3′5′H	Flavonoid 3′5′-hydroxylase

F3GT	UDP-Glc:flavonoid 3-*O*-glucosyltransferase
F3H	Flavanone 3-hydroxylase
F3′H	Flavonoid 3′-hydroxylase
GAM	Generalized additive models
GDP	Guanosine diphosphate
HCGQT	Hydroxyl cinnamoyl D-glucose: quinate hydroxycinnamoyl transferase
HCT	Hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase
HPLC	High-performance liquid chromatography
HQT	Hydroxycinnamoyl transferase
MDHA	Monodehydroascorbate
MPA	Metaphosphoric acid
MT	Methyltransferase
PAL	Phenylalanine ammonia lyase
PCA	Principal component analysis
Q-TOF	Quadrupole-time-of-flight
TAA	Total ascorbic acid
TCEP	Tris(2-carboxyethyl)phosphine
UFGT	UDP-glucose:flavonoid 3-*O*-glucosyltransferase
UGCT	UDP-glucose: cinnamate glucosyl transferase
UHPLC	Ultra high-performance liquid chromatography

LIST OF ORIGINAL PUBLICATIONS

I. Gutiérrez-Quequezana, L.; Vuorinen, A.; Kallio, H.; Yang, B. Improved analysis of anthocyanins and vitamin C in blue-purple potato cultivars. *Food Chemistry.* **2018,** 242, 217–224.

II. Gutiérrez-Quequezana, L.; Vuorinen, A.; Kallio, H.; Yang, B. Impact of cultivar, growth temperature and developmental stage on phenolic compounds and ascorbic acid in purple and yellow potato tubers. *Food Chemistry.* **2020,** 326, 126966.

III. Gutiérrez-Quequezana, L.; Vuorinen, A.; Kallio, H.; Markkinen, N.; Barua, S.; He, W.; Ollennu-Chuasam, P.; Virtanen, E.; Sipilä, A.; Tuomisto, J.; Yang, B. Effect of weather conditions affected by latitude on the content of anthocyanins, phenolic acids and ascorbic acid in potato tubers harvested at different stages of development. 2023, *Submitted*.

1 INTRODUCTION

Potato (*Solanum tuberosum* L.) is the most consumed non-cereal crop in the world. It provides our diet with an important source of carbohydrates, high quality proteins, fiber, important micronutrients such as vitamins, minerals, and phytonutrients such as carotenoids and phenolic compounds (Gibson & Kurilich, 2013; Robertson et al., 2018). Thus, this is an important crop to maintain food security and reduce malnutrition in areas where it is consumed as a staple crop. The main potato producer is China. However, the temperate zones of the Northern hemisphere concentrate the highest density of potato production. In areas around 50° N in Europe, the soil has enough moisture to produce potatoes without the need of irrigation (Haverkort et al., 2013). However, climate change is altering weather conditions all over the world. It is predicted that potato yields will be reduced by 2085 (-2 to -26 %) with high latitude areas experiencing even greater yield reductions (Raymundo et al., 2018).

There are more than 4000 potato cultivars which mostly are located in the Andes (International Potato Center, 2023). White and yellow-fleshed potatoes are the most consumed while red and purple-fleshed potatoes are still not broadly commercialized. Chlorogenic acid is the main phenolic acid in all potato cultivars. The red and purple-fleshed cultivars contain, in addition to chlorogenic acid, anthocyanins. Different studies suggest that the consumption of pigmented potatoes might improve human health (Bontempo et al., 2013; Jokioja et al., 2020; Madiwale et al., 2012; Vinson et al., 2012). The content of ascorbic acid in potato tubers ranges from 15 to 37 mg per 100 g FW (Finlay et al., 2003). Although the content of ascorbic acid in the tubers is lower than in fruits or other vegetables, the consumption of potato as a staple crop makes it an important contributor to the recommended daily intake (RDI: 75 mg ascorbic acid/day).

The content of phenolic compounds and ascorbic acid in potato depends mainly on the genotype, but the stage of tuber development and weather conditions also impact the content of these compounds. The content of phenolic acids, anthocyanins and ascorbic acid has been determined in a high number of potato cultivars (Finlay et al., 2003; Jansen & Flamme, 2006; Pillai et al., 2013; Valcarcel et al., 2016). However, there is scarce information about how the stage of tuber development and weather conditions affect the synthesis of these compounds. Most of the studies concerning the effect of weather conditions on the content of phenolic compounds and ascorbic acid have been performed under controlled conditions by evaluating the response to a single weather variable. Thus, the results can differ considerably from field experiments.

The synthesis of phenolic compounds tends to increase when plants are exposed to low but non-freezing temperatures (Schulz et al., 2015). Plants grown in temperate regions such as high latitudes have shown to produce fruits with higher content of phenolic compounds (Åkerström et al., 2010; B. Yang et al., 2013; W. Yang et al., 2016). Solar radiation, day-length, light quality, and rainfall are also important weather variables that affect the synthesis of these compounds (Jaakola & Hohtola, 2010; Samkumar et al., 2021; Tapia et al., 2022).

Weather conditions in Finland are influenced by the Atlantic Ocean. In summer, Finland has long days, and warm and cool temperatures during the day and night, respectively. Therefore, there are good environmental conditions for potato production during summer. These unique characteristic of the weather in Finland have important effects on the synthesis of phenolic compounds and other antioxidants in plants.

The main aim of this study was to understand how the stage of tuber development and weather conditions affect the expression levels of the genes involved in the phenylpropanoid pathway, and the content of anthocyanins, phenolic acids and ascorbic acid in potato cultivars grown at different latitudes in Finland. In the initial stage of the study, the extraction methods of phenolic compounds and ascorbic acid had to be improved because potato has oxidative enzymes and their effect on the stability and extraction of the analytes of interest had to be minimized.

The literature review of the thesis summarizes the latest findings about the chemical characteristics of the metabolites studied, as well as on the biosynthetic pathways for the synthesis of these compounds. The review also covers the effect of cultivar, stage of tuber development and weather conditions on the synthesis and accumulation of anthocyanins, phenolic acids and ascorbic acid in potato tubers.

2 REVIEW OF THE LITERATURE

2.1 The potato

2.1.1 Potato origin, domestication and expansion

Potato (*Solanum tuberosum* L.) is native to the Andes of South America and was domesticated 8000–10000 years ago from wild species native to the Andes of Peru (Hardigan et al., 2017). Potato starch remains found in grinding tools dated from 3400 BC to 1600 BC in Jiskairumoko (an ancient Peruvian village) showed the early use of potato and support the hypothesis that potatoes were domesticated in the Andes (Rumold & Aldenderfer, 2016).

Potato domestication was achieved through selection of wild potato species leading to landraces and after breeding to cultivars. A study on the impact of domestication on genome diversity of potato showed that the main markers of selection were observed in the genes controlling the carbohydrate metabolism, biosynthesis of glycoalkaloids, the shikimate pathway, the cell cycle, and the circadian rhythm (Hardigan et al., 2017).

Good adaptability was essential for the geographic expansion of the potato crop. Some potato cultivars were able to tuberize under the long days (16–h days) in the southern latitudes of Chile which made their later cultivation in Europe possible (Hardigan et al., 2017). This control/regulation of tuberization under long days has been identified due to the presence of a transcription factor (Cycling DOF FACTOR1-St CDF1) that unblocks the SELFPRUNING 6A pathway (Gutaker et al., 2019).

Recent genetic studies suggest that the first potatoes introduced to Europe in the sixteenth century were tetraploid Andean landraces, which mixed with the Chilean genotypes by the end of the eighteenth century. Later, after the potato late blight epidemic, Andean potatoes were re-introduced to build resistance to plant pathogens especially to the one caused by *Phytophthora infestans* (Gutaker et al., 2019).

Widespread cultivation of potatoes is due to their high nutritional value. Potato is one of the most efficient crops on the planet based on its higher yield per hectare (e.g., 22 versus 6 tons/hectare in potato and maize, respectively) (FAOSTAT, 2023); and its higher carbohydrate production per hectare compared to other staple crops such as maize, rice or wheat (Kreitzman et al., 2020).

2.1.2 Potato plant morphology and tuber development

Potato plant grows from a normal-sized seed tuber or seed piece of a ‘normal’ physiological age, harvested a few weeks after haulm destruction and stored at 2–4 °C for about 8 months until planting in the next year (Struik, 2007a). Potato plants consist of stems, stolons and tubers. Stems are usually green but can have red or purple pigmentation. Stolons are stems that grow horizontally and they may or may not form tubers (**Fig. 1**). If stolons are not covered with soil, they will form vertical stems. A tuber is a modified stem and constitutes the main storage organ of potatoes (Huaman, 1986) Thus, a tuber is the swollen part of a subterranean stolon which contains high levels of starch and proteins. Potato tubers vary in shape and texture, as well as the color of the flesh and skin.

In areas with cool and short growth season, pre-sprouting of seed potato tubers is necessary to speed up plant development and increase yields (Johansen & Mølmann, 2017). Once the seed tuber is planted, several main stems will develop. These stems will share resources from the same seed tuber at the beginning of their development but will quickly become independent units and compete with light, water, and nutrients with the other stems (Struik, 2007a).

Tuber formation in potato depends on genotype and environmental factors such as nitrogen level, temperature and light. For instance, some potato species need short day conditions to tuberize such as Solanum demissum and some lines of *Solanum tuberosum* subsp. *andigena*. Therefore, photoperiod affects tuberization of all potato species (Martínez-García et al., 2001).

The life cycle of a potato tuber comprehends tuber induction, initiation, enlargement, dormancy, and sprouting (Fernie & Willmitzer, 2001). Potato plants start to produce stolons very early after emergence or even before the shoots start to emerge. Tuberization starts parallel to stolon formation, before all stolons have been formed (Struik, 2007a).

Tuber induction starts under conditions of cool night temperatures and short days. Under these conditions, underground horizontal elongation of the stolon ceases, and the stolon tip begins to swell to form the tuber (Hannapel, 2007). During tuber induction, the main site of perception of the photoperiodic signal is in the leaves. From there, a graft-transmissible substance is transported from the leaves to stolon tips to activate tuber formation (Hannapel, 2007). Tuber induction is accompanied by biochemical changes. GA 2-oxidase gene is upregulated during the early stages of potato tuber development prior to visible swelling and is predominantly expressed in the subapical region of the stolon and the growing tube. This gene modifies gibberellin levels in the subapical stolon region at the onset of tuberization and allows tuber to develop and grow (Kloosterman et al., 2007), causing the content of gibberellic acid to decrease during tuber induction (Fernie & Willmitzer, 2001).

Tuber initiation and enlargement occurs in the early stage of tuber formation (up to 0.8 cm diameter), when cells in the pith and cortex enlarge and then divide longitudinally. Longitudinally division stops when tuber reaches 0.8 cm diameter but cell enlargement in the pith and cortex continues throughout the whole process of tuber development (Xu et al., 1998). Cell division and expansion leads to an accelerated accumulation of starch and storage proteins such as patatins (glycoproteins). These changes are a result of a coordinated expression of the genes involved in the synthesis of starch and proteins (Struik, 2007a; Viola et al., 2001). During tuber enlargement, potato tubers also decrease their general metabolic activity and behave as storage organs (Fernie & Willmitzer, 2001). Dormancy and sprouting are processes that occur simultaneously to tuber initiation and enlargement (Fernie & Willmitzer, 2001).

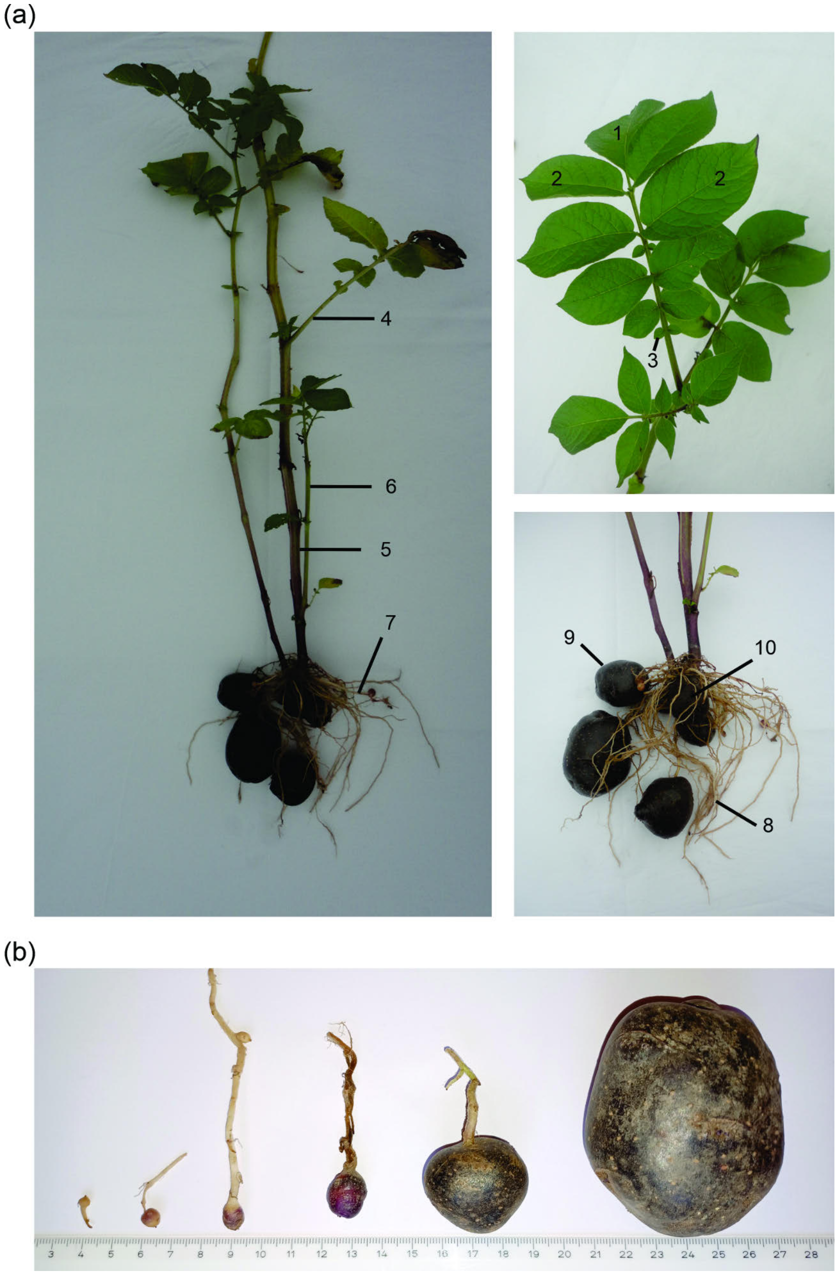


Fig. 1 (a) Potato plant morphology of cultivar ‘Synkeä Sakari’: 1) Terminal leaflet, 2) lateral leaflet, 3) interjected leaflet, 4) petiole, 5) main stem, 6) lateral stem, 7) stolon, 8) roots, 9) tuber, 10) mother tuber. (b) Stages of tuber development from subapical swelling stolon to mature tuber of a single plant of cultivar ‘Synkeä Sakari’.

2.2 Biosynthesis of anthocyanins, phenolic acids and ascorbic acid in plants

2.2.1 Biosynthesis of anthocyanins

Anthocyanins and phenolic acids are synthesized through the phenylpropanoid pathway in the cytosol at the outer surface of the smooth endoplasmic reticulum (Landi & Tattini, 2015). This starts with the deamination of phenylalanine by the enzyme phenylalanine ammonia lyase (PAL) to produce cinnamic acid. PAL reaction is a key branching point leading to the synthesis of primary and secondary compounds. Thus, PAL is a key enzyme regulating the synthesis of many phenolic compounds (Mandal et al., 2010; Marchiosi et al., 2020). Cinnamic acid is then transformed to *p*-coumaric acid by cinnamate 4-hydroxylase (C4H). *p*-Coumaric acid is conjugated with coenzyme A to produce *p*-coumaroyl CoA by the action of 4-coumarate-CoA ligase (4CL). The synthesis of *p*-coumaroyl CoA is a critical step since it divides the phenylpropanoid pathway into two routes: the flavonoid pathway and the chlorogenic acid pathway (Chaves-Silva et al., 2018).

The enzymes involved in the biosynthesis of flavonoids belong to the families 2-oxoglutarate-dependent dioxygenases, cytochromes P450 and glucosyltransferases. The flavonoid pathway (**Fig. 2**) begins with the reaction of *p*-coumaroyl-CoA with three molecules of malonyl-CoA to produce naringenin chalcone (a tetrahydroxy chalcone) using chalcone synthase (CHS) as catalysator. Naringenin chalcone is isomerized by chalcone isomerase to the three cyclic (2S)-flavone backbone producing the colorless naringenin. C-3 hydroxylation of naringenin by flavanone 3-hydroxylase leads to the production of dihydrokaempherol, which is the substrate for flavonoid 3′-hydroxylase (F3′H) or flavonoid 3′5′-hydroxylase (F3′5′H) to produce dihydroquercetin or dihydromyricetin, respectively.

The starting point for the anthocyanin pathway is when dihydromyricetin, dihydrokaempherol and dihydroquercetin are reduced by dihydroflavonol 4-reductase (DFR) and converted to the leucoanthocyanidins: leucodelphinidin, leucopelargonidin and leucocyanidin, respectively. Anthocyanin synthase (ANS) oxidizes the leucoanthocyanidins to produce the coloured anthocyanidins (flavylium ion) (Chaves-Silva et al., 2018; Tanaka et al., 2008).

The unstable anthocyanidins are further glycosylated by the uridine diphosphate-sugar-dependent glycosyltransferases (UGTs) superfamily, which transfers a sugar residue to the aglycone and are very specific (Hsu et al., 2017). For example, UDP-glucose:flavonoid 3-*O*-glucosyltransferase (UFGT) transfers a sugar residue at 3-*O*-position (Martens et al., 2010) and anthocyanin 5-*O*-glucosyltransferase (5-GT) transfers the sugar at 5-*O*-position (Yamazaki et al., 2002). One of the genes encoding UFGT is anthocyanidin 3-*O*-glysosyltransferase (*F3GT*) responsible for the glycosylation of anthocyanins in petunia (Cheng et al., 2014; Yamazaki et al., 2002). In roses, a double glycosylation was found to be produced first at 5-OH and then at the 3-OH group by UDP-glucose: anthocyanidin 5,3-*O*-glucosyltransferase (Ogata et al., 2005). Glycosylation of anthocyanins plays an important role in the stabilization of these pigments. The glycosylated anthocyanins can already be stored in the acidic environment of the vacuole or continue further glycosylation, acylation, and methylation depending on the plant family and species.

In *Petunia hybrida* it was found that rhamnosylation of anthocyanidin-3-glucosides was catalyzed by UDP rhamnose: anthocyanidin-3-glucoside rhamnosyl-transferase (3RT) adding a rhamnose at position 3 of the glucose (Brugliera et al., 1994; Kroon et al., 1994).

Anthocyanidin-3-*O*-rutinosides can be acylated, 5-*O*-glucosylated and methylated following that sequential order caused by their efficiency in using the substrates (Luo et al., 2007; Yamazaki et al., 2002).

Production of acylated anthocyanins requires the action of BAHD acyltransferase family. BAHD family comprises aromatic and aliphatic acyltransferases. Two aromatic acyltransferases have been cloned and characterized functionally (Gt5AT: Hydroxycinnamoyl CoA:anthocyanin 5-*O*-glucoside-6‴-*O*-acyltransferase, and Pf3AT: Hydroxycinnamoyl CoA:anthocyanin 3-*O*-glucoside-6″-*O*-acyltransferase) (Luo et al., 2007). BAHD enzymes have a broad substrate specificity. For example, Gt5AT (purified from *Gentiana trifloral*) catalyzed the addition of *p*-coumaric acid and caffeic acid to the 5-glucosyl moiety of delphinidin or cyanidin 3,5-diglucosides but no other types of anthocyanins (Fujiwara et al., 1997). The authors also found that Gt5AT had higher affinity to caffeoyl-CoA than to coumaroyl CoA.

Methyltranferases (MT) are responsible for the production of diverse methylated flavonoids. S-adenosyl-methionine (SAM) methyltransferases are the major class of MTs and they use SAM as a methyl donor. *O*-methyltransferase (OMT) is one subgroup of SAMs and they act as hydroxyl group acceptors. In anthocyanins, OMTs show substrate specificity for glycosylated substrates (Y. Liu et al., 2022). It was found that anthocyanin *O*-methyltransferase (AOMT) catalyze the methylation at 5′ and 3′ position of the B ring of anthocyanin 5-*O*-glucosides and has more preference for the most labile anthocyanin substrate (delphinidin-3-*O*-glucoside) (Lücker et al., 2010). Recently, it was found that methylation of anthocyanins in potato tubers is caused by *OMT30376* gene (H. Zhang et al., 2022).

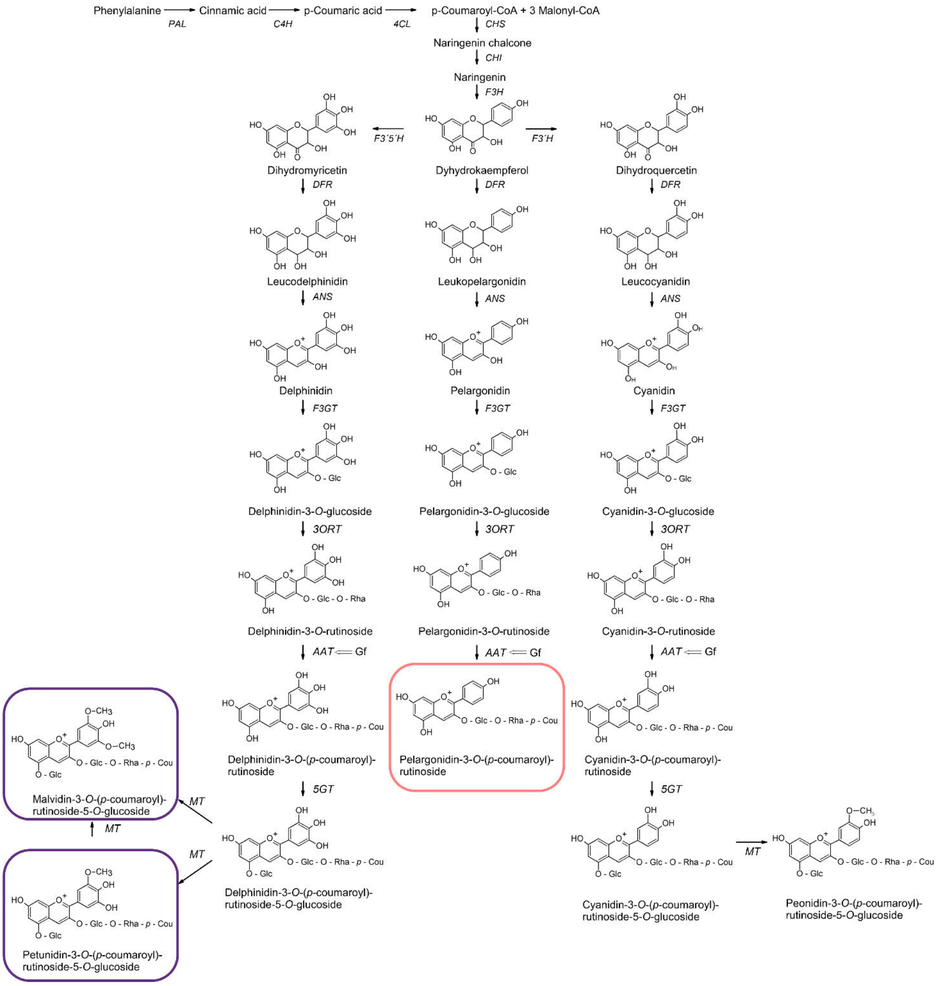


Fig. 2 Schematic diagram of anthocyanin biosynthesis pathway. Abbreviations: PAL: Phenylalanine ammonia-lyase, C4H: Cinnamate 4-hydroxylase, 4CL: 4-coumarate: CoA ligase, CHS: Chalcone synthase, CHI: Chalcone isomerase, F3H: Flavanone 3-hydroxylase, F3′H: Flavonoid 3′-hydroxylase, F3′5′H: Flavonoid F3′,5′-hydroxylase, DFR: Dihydroflavonol 4-reductase, ANS: Anthocyanidin synthase, F3GT: UDP-Glc:flavonoid 3-*O*-glucosyltransferase, 3ORT (or RT): UDP-rhamnose anthocyanidin-3-glucoside rhamnosyltransferase, MT: Methyltransferase (MT used in this study was AOMT3: flavonoid 3′,5′-methyltransferase-like), Gf is the genetic loci controlling the acylation of anthocyanidin 3-*O*-rutinoside with *p*-coumaric acid, AAT: Anthocyanin acyltransferase, 5GT: UDP-glucose:anthocyanin5-*O*-glucosyltransferase. (Chaves-Silva et al., 2018; Yamazaki et al., 2002).

2.2.2 Biosynthesis of phenolic acids

The synthesis of chlorogenic acids starts in the core phenylpropanoid pathway. The main chlorogenic acid in potatoes is 5-*O*-caffeoylquinic acid but other hydroxycinnamic acids, such as caffeic acid and ferulic acid among others, are also found in minor amounts. The biosynthetic pathway leading to the synthesis of these phenolic acids is still not very clear. There are three possible routes proposed for the synthesis of chlorogenic acid.

Two of these routes derive from *p*-coumaroyl-CoA known as the "shikimate shunt" and the "quinate shunt" (Volpi e Silva et al., 2019). As mentioned before, the synthesis of *p*-coumaroyl-CoA is crucial to produce diverse phenolic compounds such as flavonoids, coumarins, stilbenes, proanthocyanidins, even lignin (a cell wall phenolic biopolymer which is produced by the "shikimate shunt") among others. (Hu et al., 2022; Vogt, 2010).

The "shikimate shunt", suggests that hydroxycinnamoyl-CoA:shikimate hydroxycinnamoyl transferase (HCT) catalyzes the reaction of *p*-coumaroyl-CoA with shikimic acid to produce *p*-coumaroyl shikimic acid. The last one is then hydroxylated by *p*-coumaroyl shikimate 3′-hydroxylase (C3′H) producing caffeoyl shikimic acid which is then de-esterified to caffeoyl-CoA. Hydroxycinnamoyl-CoA quinate hydroxycinnamoyl transferase (HQT) re-esterifies caffeoyl-CoA and quinic acid to produce chlorogenic acid (5-CQA) (Route I, **Fig. 3**). It is suggested that this is the main route in some *Solanaceous* species such as tomato and tobacco (Niggeweg et al., 2004).

In the "quinate shunt", *p*-coumaroylquinic acid reacts with quinic acid via HCT or HQT to form *p*-coumaroylquinic acid. It has been demonstrated that quinic acid has higher affinity to HQT than HCT in artichoke (Sonnante et al., 2010), but more studies need to be performed to identify the enzyme that is involved in this reaction. Finally, *p*-coumaroylquinic acid is hydroxylated by C3′H to produce chlorogenic acid (5-CQA) (Route II, **Fig. 3**).

A positive correlation was shown between HQT expression levels and chlorogenic acid content in tomato (Clé et al., 2008). Similarly, a study with twelve potato cultivars indicated that HQT transcripts correlated well with chlorogenic acid levels, while the HCT transcript levels showed a negative correlation with chlorogenic acid levels. Thus, the authors suggested that Route II (**Fig. 3**) would be the main route for the synthesis of chlorogenic acid in potato tubers (Valiñas et al., 2015). In the same study caffeic acid correlated well with chlorogenic acid and with caffeoyl shikimate esterase (CSE) (Route I, **Fig. 3**) suggesting that this would be the main enzyme leading to the synthesis of caffeic acid in potato tubers. However, a study in model plants showed that 4-coumarate 3-hydroxylase (C3H, an ascorbate peroxidase) catalyzes the hydroxylation of *p*-coumaric acid to caffeic acid (Barros et al., 2019).

In sweet potato (*Ipomea batatas*) it has been found that chlorogenic acid is synthesized through the conversion of cinnamic acid to cinnamoyl D-glucose by UDP-glucose: cinnamate glucosyl transferase (UGCT). Later, two hydroxyl groups are attached to the benzene ring of cinnamoyl D-glucose to form caffeoyl D-glucose. Finally, hydroxyl cinnamoyl D-glucose: quinate hydroxycinnamoyl transferase (HCGQT) mediates the esterification of caffeoyl D-glucose with quinic acid to yield chlorogenic acid (5-CQA) (Route III, **Fig. 3**) (Niggeweg et al., 2004; Villegas et al., 1987).

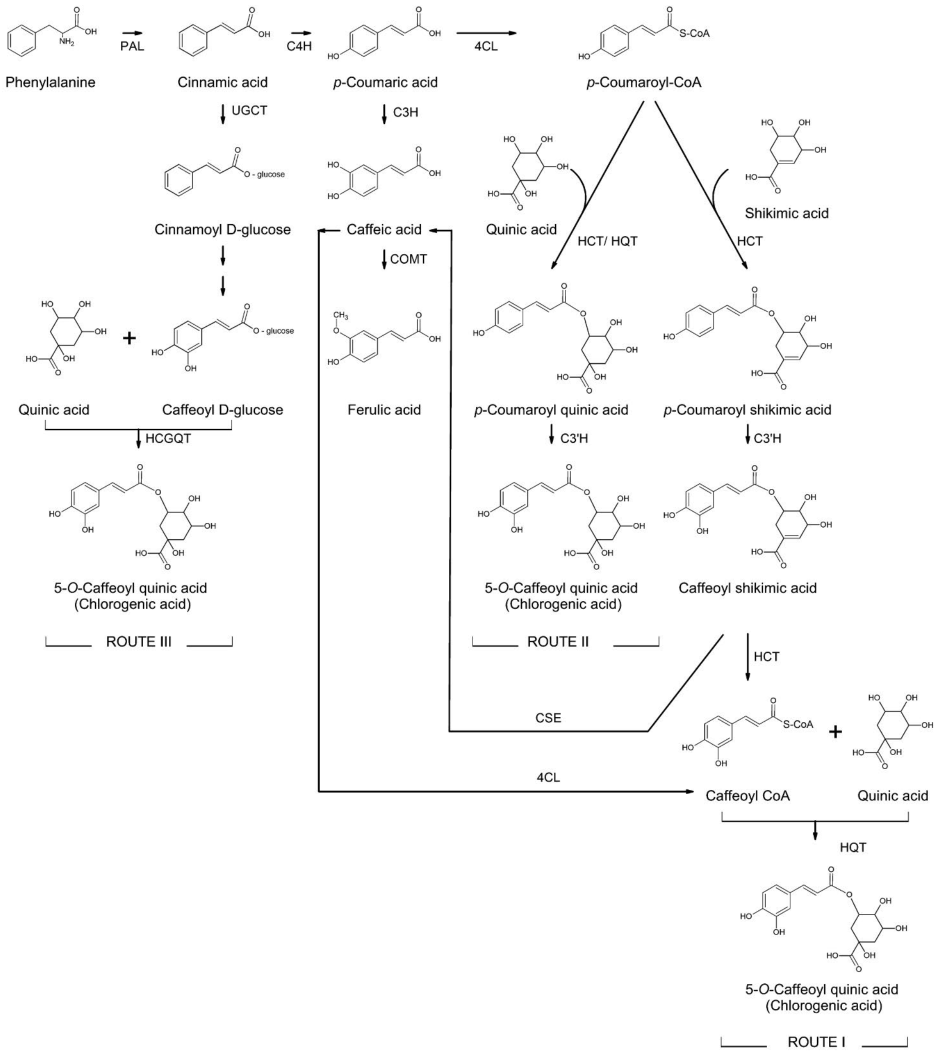


Fig. 3 Schematic diagram of chlorogenic acid biosynthesis pathway. (Barros et al., 2019; Niggeweg et al., 2004; Valiñas et al., 2015; Volpi e Silva et al., 2019).

2.2.3 Biosynthesis of ascorbic acid

Ascorbic acid (ascorbate or vitamin C) is synthesized by eukaryotes except for primates and some other animal groups. These groups of animals have lost the ability to synthesize ascorbic acid because the gene for L-gulono-1,4-lactone oxidase (the last enzyme needed for the synthesis of ascorbic acid in animals) has suffered mutations not allowing it to synthesize ascorbic acid (Padayatty & Levine, 2016; Smirnoff, 2018). Animals, green plants and photosynthetic protists synthesize ascorbic acid through different pathways.

Studies performed on *Arabidopsis thaliana* have shown that the main pathway for the synthesis of ascorbic acid in plants is through the D-mannose/ L-galactose pathway (also known as the Smirnoff-Wheeler pathway) and in other plant species via the uronic acid precursors (Bulley et al., 2012; Conklin et al., 1999; Smirnoff, 2018; Smirnoff et al., 1998). In potato tubers the synthesis of ascorbic acid through the Smirnoff-Wheeler pathway was confirmed by using transgenic potato plants with reduced guanosine diphosphate (GDP)-mannose pyrophosphorylase activity. The tubers of the transgenic plants had lower ascorbic acid than wild-type potatoes (Keller et al., 1999).

In land plants, the Smirnoff-Wheeler pathway (**Fig. 4**) does not involve inversion of the carbon chain (thus, C1 of D-glucose is transformed to C1 of L-ascorbate) (Wheeler et al., 2015). D-mannose and L-galactose are precursors for ascorbic acid biosynthesis. The synthesis of ascorbic acid starts with the conversion GDP-D-mannose to GDP-L-galactose by a double epimerization catalyzed by GDP-D-mannose-3′,5′-epimerase. GDP-L-galactose phosphorylase breaks GDP-L-galactose into free L-galactose which is later oxidized to L-galactono-1,4-lactone by a NAD^+ dependent L-galactose dehydrogenase. All these previous steps take place in the cytosol. Finally, oxidation of L-galactono-1,4-lactone to L-ascorbate by L-galactono-1,4-lactone dehydrogenase is carried out in the mitochondria using cytochrome c as an electron acceptor thus avoiding production of H_2O_2 (Smirnoff, 2018; Smirnoff et al., 1998).

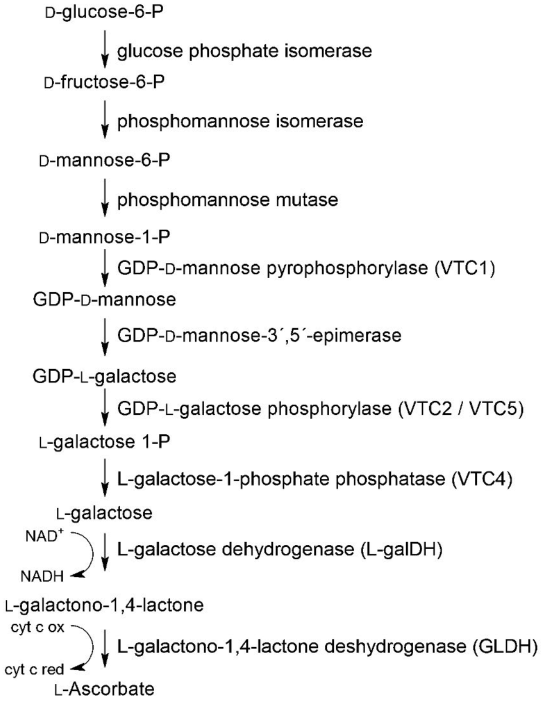


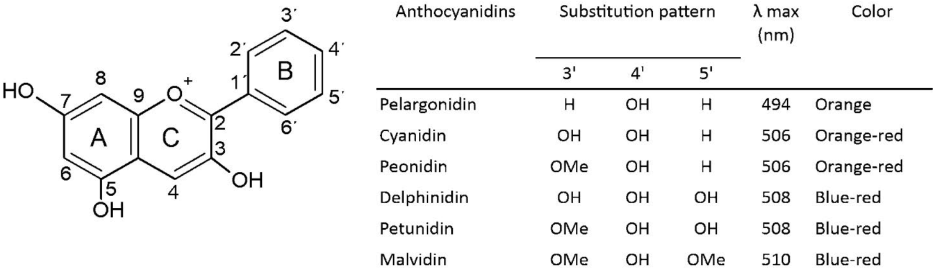
Fig. 4 Smirnoff-Wheeler pathway for the biosynthesis of ascorbate in plants (Smirnoff, 2000, 2018; Wheeler et al., 2015).

2.3 Chemical properties of anthocyanins, phenolic acids and ascorbic acid

2.3.1 Anthocyanins

Anthocyanins are plant pigments responsible for the red, blue, and purple hues in different plant organs such as leaves, fruits, flowers, roots, and tubers.

Anthocyanins are glycosylated flavonoids composed by the diphenyl-propane backbone ($C_6C_3C_6$) which consists of a benzene ring (A) connected to a pyran ring (C) which is linked to a phenyl ring (B) (**Fig. 5**). They are derivatives of the 2-phenylbenzopyrilium cation (flavylium cation). The main structure of anthocyanins consists of an aglycone (anthocyanidin) and a sugar(s). Thus, anthocyanins are water-soluble glycosides of anthocyanidins. Anthocyanins can contain one, two or three saccharide units. The most common monosaccharides linked to the anthocyanidins are glucose, galactose, rhamnose, arabinose, xylose, and glucuronic acid; and the most common disaccharides are rutinose, sophorose, sambubiose, gentiobiose, etc. The sugar moiety is connected to the anthocyanidin by an *O*-linkage. This sugar moiety is usually located at 3-position, if there is a second sugar this is usually located at the 5-hydroxyl position, but it can also be located at 7-,3′-, or 5′- position (Alseekh et al., 2020; Andersen & Jordheim, 2010).



Anthocyanidins	Substitution pattern			λ max (nm)	Color
	3'	4'	5'		
Pelargonidin	H	OH	H	494	Orange
Cyanidin	OH	OH	H	506	Orange-red
Peonidin	OMe	OH	H	506	Orange-red
Delphinidin	OH	OH	OH	508	Blue-red
Petunidin	OMe	OH	OH	508	Blue-red
Malvidin	OMe	OH	OMe	510	Blue-red

Fig. 5 Basic structure of anthocyanins – the flavylium cation (Andersen & Jordheim, 2010; Giusti & Wrolstad, 2003).

The variety of anthocyanins is based on a) the degree of B-ring hydroxylation and/or methoxylation, b) the type, number, and site of attachment of the sugars to the anthocyanidin, and c) the type, number and linkage position of the acyl groups attached to the sugars. For instance, the brilliant blue petal flowers commonly contain polyacylated anthocyanins forming complexes with metals (Mori et al., 2006). Anthocyanin-metal ion complexes known as metalloanthocyanins are produced by the conjugation of anthocyanins with ions of Al, Cd, Cu, Zn, Fe, Mo, W, among others (Landi & Tattini, 2015).

Both aliphatic and aromatic acids appear as acyl groups of anthocyanins. The most common aromatic acids are phenolic acids including hydroxycinnamic acids (e.g., *p*-coumaric acid, caffeic acid, ferulic acid, sinapic acid), and hydroxybenzoic acids (e.g., *p*-hydroxybenzoic acid, gallic acid). Common aliphatic acids are e.g., malonic acid, acetic acid and succinic acid. The organic acid in anthocyanins is esterified to the sugar (Vermerris & Nicholson, 2006). The major acids in potato anthocyanins are *p*-coumaric acid and ferulic acid. The first anthocyanin isolated from potato was obtained from the purple cultivar ‘Negrese’ (also known as ‘Congo’). This anthocyanin was denominated petanin (3-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-α-rhamnopyranosyl)-β-glucopyranoside]-5-*O*-β-glucopyranoside) (Chmielewska, 1936).

Color and stability of anthocyanins depend on the degree of hydroxylation or methylation of the B-ring, and by the type and degree of glycosidation and acetylation. For example, in a previous study the *cis*-*trans* stereochemistry of the acylated groups was found to affect the spectra, color, and stability of anthocyanins. *Cis* acylation increased the λmax absorbance and thus increased the color intensity, while *trans* acylation increased stability over time at pH > 1 for delphinidin and petunidin derivatives (Sigurdson et al., 2018).

Also, external factors such as the pH of the medium influence the chemical structure of anthocyanins. At pH below 2, anthocyanins exist mainly in the form of flavylium cations. When the pH increases (from 2 to 4) the flavylium cation loses a proton and produces the quinoidal base. Parallelly, hydration of the flavylium cation produces the colorless carbinol pseudobase which slowly equilibrates to the open chalcone form. The amounts of the flavylium cation, the quinoidal base, the colorless carbinol pseudobase or the open chalcone form, depend on the anthocyanin structure and pH. In the case of malvidin 3-glucoside, when exposed to pH 4 to 6, the color disappears as the predominant form is the carbinol pseudobase (Mazza & Brouillard, 1987).

Thermal degradation of anthocyanin has also been studied in different vegetables and under different heating treatments and storage conditions (Cisse et al., 2009; Patras et al., 2010). Thermal degradation of anthocyanins follows a first order kinetic reaction (Dyrby et al., 2001; Hellström et al., 2013; Reyes & Cisneros-Zevallos, 2007; Slavu et al., 2020), and that the initial product of the degradation is the colorless chalcone (Furtado et al., 1993). Other products formed during thermal anthocyanin degradation are anthocyanidins, 2,4,6-trihydroxybenzaldehyde, 2,4,6-trihydroxy-benzoic acid, 3,4,5-trihydroxybenzoic acid, 3,4-dihydroxy-5-methoxybenzoic acid among others (Furtado et al., 1993; Sharma et al., 2016).

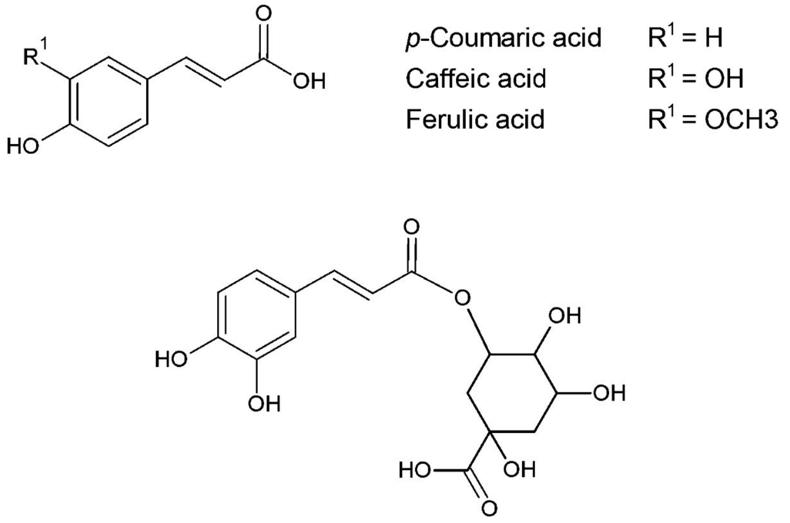
Although the stability of the anthocyanins depends on the pH of the medium, co-pigmentation with other compounds also plays an important role. The main co-pigments are mainly flavonoids (including the anthocyanins themselves), phenolic acids, hydrolyzable tannins and, to a lesser extent, alkaloids, amino acids, nucleotides and polysaccharides (Trouillas et al., 2016). These compounds produce a bathochromic shift (increment of wavelength) and hyperchromic effect (increment of the absorbance of the UV band). Co-pigmentation is affected by the pH of the medium, temperature and the ratio between the concentration of anthocyanins and co-pigment. (Eiro & Heinonen, 2002; Mazza & Brouillard, 1987). One of the initial studies on understanding the mechanisms of anthocyanin co-pigmentation, specifically self-association, demonstrated a vertical stacking structure caused by π–π interaction for the self-association of anthocyanins (Hoshinot et al., 1982). Later, other mechanisms such as intermolecular (Brouillard et al., 1989), intramolecular (Dangles et al., 1993; Figueiredo et al., 1999), and metal co-pigmentation (Kondo et al., 1992) were demonstrated. Anthocyanin-metal complex is an interesting case: red roses and blue cornflowers possess the same anthocyanin (protocyanin), but association with different metal ions changes the color of the petals to red in roses and blue in cornflowers. X-ray crystallographic analysis showed that the structure of protocyanin in blue cornflowers contained six molecules of anthocyanin cyanidin-3-*O*-(6-*O*-succinyl glucoside)-5-*O*-glucoside stacked with six molecules of flavone apigenin 7-*O*-glucuronide-4-*O*-(6-*O*-malonyl-glucoside) and four metal ions (Fe^{3+}, Mg^{2+} and two Ca^{2+}). It is evident that association of this metalloanthocyanin with flavones and Ca^{2+} ions modify the co-pigmentation and stability of the molecule (Shiono et al., 2005).

In purple-fleshed potatoes, the most common anthocyanins are methylated delphinidin derivatives (petunidin or malvidin), while in red-fleshed potatoes pelargonidin derivatives predominate. Pelargonidin derivatives extracted from red-fleshed potato tubers showed higher stability at pH 3 than petunidin derivatives extracted from purple-fleshed tubers (Reyes & Cisneros-Zevallos, 2007). Potato anthocyanins are usually mono-acylated with hydroxycinnamic acids (coumaric, ferulic or caffeic acid) showing a secondary absorption band at around 315 nm (Rodriguez-Saona et al., 1998). Acylation of anthocyanins protects the central chromophore from nucleophilic attack of water molecules at position 2 and 4 of the aglycone due to intramolecular co-pigmentation between the acyl residues and the chromophore. (Figueiredo et al., 1999). Thus, the acylated potato anthocyanins might be more stable over a longer pH range than the non-acylated anthocyanins.

2.3.2 Phenolic acids

Phenolic acids can be classified as hydroxybenzoic acids and hydroxycinnamic acids. They vary according to the hydroxylation or methoxylation of the phenol ring. Hydroxybenzoic acids have a carboxylic group substituted on a phenol and have the structure C6-C1 (e.g. *p*-hydroxybenzoic acid, gallic acid, vanillic acid), while hydroxycinnamic acids have the C6-C3 structure (e.g. cinnamic acid, *p*-coumaric acid, caffeic acid, ferulic acid) (**Fig. 6**). Phenolic acids in plants are present as simple esters of glucose, tartaric acid or quinic acid. Hydroxybenzoic acids are usually present as glucosides and esters with carbohydrates, while hydroxycinnamic acids are usually esterified with tartaric acid or quinic acid. Caffeic acid is one of the most common hydroxycinnamic acids in plants and can occur as chlorogenic acid (an ester of caffeic acid with quinic acid) (Vermerris & Nicholson, 2006).

Around 40 phenolic compounds have been characterized in potato tubers, with hydroxycinnamic acids being an important part. The main phenolic acid in potato is 5-*O*-caffeoylquinic acid (chlorogenic acid) which represents approximately 80–90 % of the phenolic acids. Additionally, other caffeoyl quinic acids such as 3-*O*-caffeoylquinic acid (neochlorogenic acid), and 4-*O*-caffeoylquinic acid (cryptochlorogenic acid), unconjugated hydroxycinnamic acids, such as caffeic acid, and *p*-coumaric acid, feruloyl quinic acids and hydroxycinnamic acids linked to amino compounds have been found in potato tubers but in minor amounts (Giusti & Wrolstad, 2001; López-Cobo et al., 2014; Navarre et al., 2011).



5-O-Caffeoyl quinic acid
(Chlorogenic acid)

Fig. 6 Structures of some phenolic acids in potato tubers.

2.3.3 Ascorbic acid

Ascorbic acid is a polar compound and a 2,3-enediol with acidic and reducing properties (**Fig. 7**). Delocalization of the π-electrons on the conjugated enediol causes ionization of the C3 hydroxyl group making it very acidic and dissociating at pKa 4.13. A second ionization occurs at C2 hydroxyl group and takes place at pKa 11.6 (Davey et al., 2000).

At physiological pH (7.35–7.45), L-ascorbic acid is present as a monovalent ion known as L-ascorbate. When the first electron of L-ascorbate is lost, it produces the radical monodehydroascorbate (MDHA) also known as ascorbate radical or semidehydroascorbate. When the second electron is lost, it causes oxidation of MDHA to dehydroascorbic acid (DHA), which exists as hydrated and anhydrous forms (Padayatty & Levine, 2016). In plants, ascorbate reacts with H_2O_2 in a reaction catalyzed by ascorbate peroxidase (APX) leading to the production of MDHA. Later, MDHA and DHA can be reversibly reduced to ascorbate by NADH and GSH-dependent enzymes, respectively. MDHA does not react with oxygen or other molecules to produce reactive radicals and therefore it is an effective radical scavenger (Smirnoff, 2018). DHA is unstable and can be hydrolyzed, causing irreversible cleavage of its ring, producing 2,3-diketogulonic acid (Padayatty & Levine, 2016).

In plants, ascorbic acid is present mainly as L-ascorbic acid (the reduced form) and DHA is present in minor amounts. It was previously thought that DHA was formed during the extraction of ascorbic acid. However, it has been demonstrated that DHA is formed *in vivo*, and that plant cells have a specific transporter for DHA (Foyer & Mullineaux, 1998; Horemans et al., 2008).

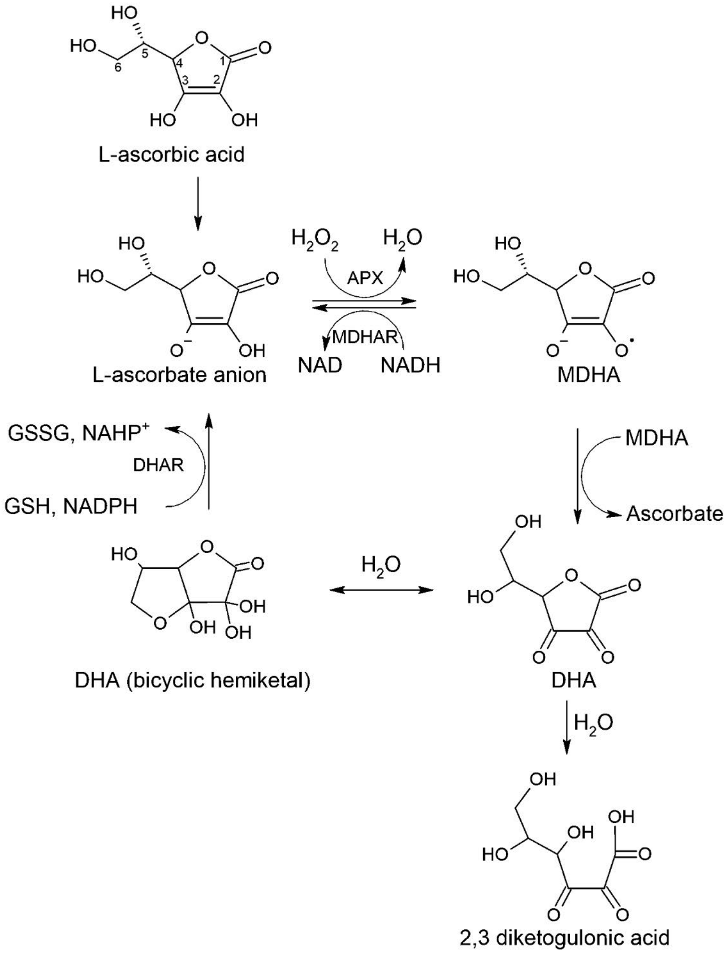


Fig. 7 Redox reactions of ascorbic acid (Padayatty & Levine, 2016; Smirnoff, 2000, 2018).

2.4 Role of anthocyanins, phenolic acids and ascorbic acid in plants

2.4.1 Role of anthocyanins in plants

An important function of anthocyanins is to protect plant tissues against oxidative stress caused by abiotic stressors, which can damage e.g., DNA, proteins, and lipids. A study on *Brassica rapa* characterized different *DFR* genes and observed that the expression of certain *DFR* genes showed positive correlation with anthocyanin accumulation and had higher expression levels when subjected to cold stress (Ahmed et al., 2014). Similarly, accessions of *Arabidopsis thaliana* with better freezing tolerance had elevated content of anthocyanins and other flavonoids when subject to cold temperatures (Schulz et al., 2016).

Plants such as *Brassica napus* L. shoots growing in high salinity and drought environments overexpressed *DFR* and showed increased anthocyanin accumulation and decreased reactive oxygen species. This showed the greater tolerance of those plants to grow in the harsh environment (Kim et al., 2017).

Anthocyanins act also as sunscreens protecting the plant against UV-B light. The compounds absorb a fraction of the yellow/green and ultraviolet wavelengths that reach the chloroplasts, reducing the damage to photosystem II, to DNA and to the other photosynthetic apparatuses. The annual plant *Silene littorea,* when exposed to different UV treatments, showed higher content of anthocyanins when exposed to the highest UV radiation. This was interpreted as the capacity of the plant to prevent photoinhibition and to promote rapid photosynthetic recovery. The exposure to high UV radiation caused low flower and pollen production affecting the reproduction of this species (del Valle et al., 2020). Another study evaluated the photoprotection of anthocyanins in green and purple basil leaves. It was found that when the leaves were exposed to full solar irradiation, less photo damage was produced to the structure of the purple leaves than of the green leaves. The purple leaves were characterized by the presence of coumaroyl derivatives of cyanidin (Tattini et al., 2014). Thus, anthocyanins might be able to quench H_2O_2 that enters the vacuole when exposed to high light conditions and when the activities of choloroplastic antioxidants and ascorbic acid are depleted (Landi & Tattini, 2015).

Anthocyanins can enhance metal chelation and its incorporation into the vacuoles. A study with different *Brassica rapa* species (grown in substrates with different ammonium molybdate concentrations) indicated the anthocyanin-free mutants to accumulate less molybdenum (Mo) than the anthocyanin-containing species (Hale et al., 2001). This selective sequestration of Mo improves the plant nutrient status. Mo is an essential micronutrient for plants and its deficiency causes poor growth, less chlorophyll and lower content of ascorbic acid. On the other hand, the association of anthocyanins with toxic metals (such as Cd, As, Ni, etc.) has also been observed in different plant species, and the use of these plants in soil and water phytoremediation has been studied (Guérin et al., 2022; Leão et al., 2017; F. Zhang et al., 2014). Thus, the increasing contents of anthocyanins in plants and their correlation with increasing accumulation of metal ions, supports the hypothesis that metal-chelation is an important mechanism of plant defense to withstand toxic ion stress (Landi & Tattini, 2015).

A positive role, not so well studied, of anthocyanins is their photoprotective effect on compounds that are part of the defensive mechanism of plants and are toxic to insects, bacteria and fungi e.g., thiarubrines. A study on *Ambrosia chamissonis* showed that its laticifers contained red phototoxic thiarubrines which decompose to colorless thiophenes when exposed to light. Thus, when different parts of the plant were irradiated with UV light *in vitro*, the content of thiarubrines in roots (lacking anthocyanins) decreased by 94 % after 30 min irradiation and disappeared after 4 hours of irradiation. However, the content of thiarubrine in leaves and stems (containing cyanidin derivatives) was not affected by the light irradiation (J. E. Page & Towers, 2002).

Moreover, the red coloration of fruits, leaves, stems and other plant organs caused by the presence of anthocyanins, might be perceived by predators as an aposematic coloration warning them of the toxicity of the plant (Gould et al., 2009).

In contrast, anthocyanins may also facilitate plant reproduction since the red, purple, and blue bright colors attract different pollinators and seed dispersers. A study on the preference of different pollinators on two species of monkeyflowers (*Mimulus lewisii* and *M. cardinalis*) showed that bumblebees showed preference for the pink-flowered *M. lewisii* while hummingbirds were more attracted to the red-flowered *M. cardinalis*. The petals of *M. lewisii* contained lower contents of pelargonidin derivatives but higher proportion of pelargonidin monosaccharides than the petals of *M. cardinalis* (Wilbert et al., 1997). Likewise, another study on *Petunia integrifolia* (with violet-reddish flowers) and *Petunia axillaris* (with white flowers) indicated that different flower colors attract different pollinators. White flowers were more attractive to bees and beetles during the day and to hawk moths at night, while violet-reddish flowers were more attractive to bees and butterflies during the day and received no visitors at night (Hoballah et al., 2007). Thus, anthocyanins may act synergistically with other pollinating mechanisms.

Additionally, fruits adapted for dispersal by birds are mainly red and black, usually as the result of anthocyanin production (Gould et al., 2009). For instance, it was found in a study performed in the Amazon basin that the main colors of the fruits were black, blue, and red, and coincidentally, this area has one of the richest frugivorous bird communities in the world (Sinnott-Armstrong et al., 2021). However, this seed dispersal mechanism linked to the presence of anthocyanins has not been extensively studied and needs to be validated.

2.4.2 Role of phenolic acids in plants

Phenolic acids have similar functions as anthocyanins. They act as electron carriers, produce phytohormones, attract pollinators and seed dispersers, and can produce compounds that protect plants (Marchiosi et al., 2020).

An important function of phenolic acids is that they are useful in the biosynthesis of structural components of the cell wall. Phenolic acids such as coumarins and tannins affect the permeability of the membrane and control ion fluxes and hydraulic conductivity of roots. They also have a role in phytohormone regulation e.g., ferulic acid increased the activities of peroxidase, catalase, and indoleacetic acid oxidase and decreased the activity of polyphenol oxidase in maize seedlings (Einhellig F. A., 2004).

Phenolic acids absorb light between 280 and 340 nm filtering out harmful UV-B radiation. Thus, the accumulation of chlorogenic acid has been associated with UV-tolerance in the plants (Clé et al., 2008). Likewise, chlorogenic acid levels increased when chili pepper leaves were exposed to UV-B light (Rodríguez-Calzada et al., 2019).

Phenolic acids mediate plant defense responses against pathogens and insects. Chlorogenic acid protects plants against the attack of insect herbivores (Kundu et al., 2019). It accumulated in potato leaves when the tubers were infested with *Tecia solanivora* (a tuber moth), and that increment reduced the larval attack of armyworms (*Spodoptera exigua* and *Spodoptera frugiperda*) (Kumar et al., 2016). The anti-nutritive effect of chlorogenic acid to insects was explained due to the pro-oxidant effect of the acid. Oxidized chlorogenic acid binds to dietary proteins (lysine and histidine) in the insect gut and reduces the bioavailability of amino acids and proteins reducing the growth rate of insects (Felton et al., 1992). Moreover, caffeoylputrescine and dicaffeoylspermidine also increased after herbivore attack in *Nicotiana attenuata* plants (Kaur et al., 2010).

2.4.3 Role of ascorbic acid in plants

Ascorbic acid is the most important water-soluble antioxidant in plants and is an essential metabolite for plant growth and development (Foyer et al., 2020). The redox status of L-ascorbic acid is affected by the expression of dehydroascorbate reductase (DHAR) which is responsible for regenerating ascorbic acid from its oxidized state. Plants with low expression of DHAR showed slow rate of leaf expansion and reduced foliar dry weight and premature leaf aging (Z. Chen & Gallie, 2006). In addition, the redox status of apoplastic ascorbic acid and its balance with H_2O_2 levels influenced the degree of lignification (Davey et al., 2000).

Ascorbic acid acts as an enzymatic cofactor by regulating numerous enzymatic reactions involved in plant cell growth, regulation of flowering, and synthesis of secondary metabolites. For example, prolyl 4-hydroxylases are 2-oxoglutarate-dependent dioxygenases localized in the endoplasmic reticulum which require ascorbic acid as a substrate for their optimal activity (Arrigoni & de Tullio, 2000). A previous study showed that the synthesis of hydroxyproline is strongly correlated to the availability of ascorbic acid in potato tubers slices (Arrigoni et al., 1977). Plants deficient in prolyl 4-hydroxylases showed impaired root hair elongation (Velasquez et al., 2015). Time of flowering is also regulated by ascorbic acid via the nitric oxide levels in the plant. It was found in a study performed on *Arabidopsis thaliana* that low ascorbic acid levels caused low production of nitric oxides and led to early flowering (Senthil Kumar et al., 2016). The synthesis of secondary metabolites is also dependent on the endogenous ascorbic acid levels. Studies *in vitro* observed that the conversion of flavanones to flavones and of dihydroflavonols to flavonols required 2-oxoglutarate-dependent dioxygenases, Fe^{II} and ascorbic acid as cofactors (Britsch et al., 1981; Forkmann et al., 1980). It is suggested that ascorbate increases the activity of 2-oxoglutarate-dependent dioxygenases by stabilizing Fe^{II} in the active site of the enzymes (Martens et al., 2010). A study performed with ascorbate-deficient mutants and wild types of *Arabidopsis thaliana* found a positive correlation between the accumulation of ascorbic acid and anthocyanins (which involve different 2-oxoglutarate-dependent dioxygenases for their biosynthesis). This suggested that ascorbic acid deficiency disrupts the biosynthesis of anthocyanins probably due to inactivation of the iron site of 2-oxoglutarate-dependent dioxygenases (Page et al., 2012).

Ascorbic acid also has an important role in photoprotection as cofactor in the xanthophyll cycle. In this cycle, violaxanthin de-epoxidase allows the conversion of violaxantin to antheraxantin and then to zeaxantin. Violaxanthin de-epoxidase is active at low pH (below 6.5) and requires ascorbate (in the ionized form) as reductant. Plants deficient in ascorbic acid showed reduced activity of violaxanthin de-epoxidase and consequently low non-photochemical quenching of excess light energy in photosystem II (Müller-Moulé et al., 2002).

Ascorbic acid is the major redox buffer in the apoplasts where it acts as an antioxidant (Parsons & Fry, 2012; Smirnoff, 2000). It is involved in the redox signaling network of the plant response to abiotic and biotic stress (Smirnoff, 2000, 2011). Ascorbic acid reacts with superoxide, singlet oxygen, ozone and hydrogen peroxide and removes these reactive oxygen species which are generated during aerobic metabolism and during exposure to some pollutants and herbicides (Smirnoff, 1996). The ascorbic acid redox state of a plant affects its response to the presence of reactive oxygen species caused by environmental factors such as high ozone levels, high light, high or low temperature, drought and salt stress, among others. Plants with increased ascorbate and/or dehydroascorbate reductase showed higher tolerance to these environmental stresses than plants with low levels of ascorbate or reduce capacity to recycle ascorbate (Gallie, 2013). Ascorbic acid also prevents xenobiotic toxification. Aluminum-stressed tobacco plants overexpressing monodehydroascorbate reductase showed lower hydrogen peroxide content, less lipid peroxidation and lower level of oxidative DNA damage as well as better root growth than wild-type plants (Yin et al., 2010).

In plants, ascorbic acid acts as an electron donor for photosynthetic electron transport with the aid of cytochrome b and ascorbate oxidase. Cytochrome b is a protein involved in trans-membrane electron transport and is located in the plasma membrane. Cytochrome b and ascorbate oxidase regenerate apoplastic ascorbate by catalyzing the oxidation of ascorbic acid on one side of the membrane, and the reduction of MDHA (produced by oxidation of ascorbic acid) on the other side of the membrane. It has been shown that this electron transport mechanism helps to dissipate excess light energy absorbed by the chloroplasts to be transported and dissipated in apoplasts (Nanasato et al., 2005).

2.5 Effect of potato cultivar on phenolic compounds and ascorbic acid

The content of phenolic compounds in potato cultivars has been investigated in various studies. A study performed on 74 native potato cultivars from the Andean region of South America showed the phenolic compounds to vary from 1.12 to 12.37 mg gallic acid equivalents per g DW. It was also found that the content of phenolic compounds correlated positively with the antioxidant capacity (Andre et al., 2007). Similar ranges for the content of phenolic compounds were recognized in a study with 60 potato cultivars grew in Ireland (Valcarcel et al., 2015).

Usually, potato cultivars with purple or red flesh, due to the presence of anthocyanins, tend to have two to three times higher content of phenolic compounds than the cultivars with white or yellow flesh (Lewis et al., 1998; Payyavula et al., 2013). Previous studies have shown that the content of anthocyanins in colored potatoes can vary from 0.27 to 34 mg / g DW (Pillai et al., 2013). Similar contents in purple and red-fleshed potatoes have been found by other authors (Giusti et al., 2014; Jansen & Flamme, 2006).

The anthocyanin profile depends on the potato cultivar. Red-pigmented potatoes are characterized by higher contents of pelargonidin derivatives (e.g., ‘Highland Burgundy Red’, ‘Desirée’), while the blue or purple-pigmented tubers contain higher amounts of either petunidin (‘Blue Congo’, ‘Blaue Schweden’) or malvidin derivatives (‘Blaue Veltlin’, ‘Vitelotte’) (Gutiérrez-Quequezana et al., 2020; Lachman et al., 2009; Lewis et al., 1998). Additionally, a cultivar with cyanidin derivatives as the main anthocyanins (‘British Columbia Blue’) has been reported to exist (Lachman et al., 2009).

Potato is an excellent source of phenolic acids. A study performed with 27 potato cultivars showed that the main phenolic acid was chlorogenic acid, and that there was a positive correlation between the anthocyanins and phenolic acids concentrations (Lewis et al., 1998).

The content of ascorbic acid varies independently from the color of the flesh or skin of the potato cultivar. A study performed with 74 Andean potato cultivars with different flesh and/or skin colors revealed that the content of ascorbic acid ranged from 0.22 to 0.69 mg / g DW (Andre et al., 2007). A trial of 60 potato cultivars carried out in Ireland showed that the content of ascorbic acid differed from 0.12 to 0.8 mg / g DW (Valcarcel et al., 2016). Again, another study performed on 33 potato genotypes with pale colors cultivated in three different countries in Europe indicated higher contents of ascorbic acid, between 0.66 and 1.54 mg / g freeze-dried matter (Finlay et al., 2003). Besides the type of cultivar, several other factors may have affected the content of ascorbic acid in potato tubers e.g., the agronomic practices, environmental conditions and even the analytical procedures.

2.6 Effect of tuber developmental stage on the synthesis of phenolic compounds and ascorbic acid

Only limited information is available on how the stage of tuber development regulates the synthesis of anthocyanins, phenolic acids and ascorbic acid in potato tubers. The few studies performed in this topic have shown that the content of anthocyanins and phenolic acids are high in immature tubers (baby potatoes) and decrease towards maturation (Navarre et al., 2013; Payyavula et al., 2013). The results showed the main phenolic acid to be chlorogenic acid (5-*O*-caffeoylquinic acid) which clearly decreased during tuber development, while minor hydroxycinnamic acids e.g., 3-*O*- and 4-*O*-caffeoylquinic acid increased. Similarly, the main anthocyanins in purple and red-fleshed potato cultivars (petunidin-3-*p*-coumaroylrutinoside-5-glucoside and pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside, respectively), decreased during tuber development, while minor anthocyanins such as cyanidin and delphinidin derivatives showed steady amounts during tuber development (Payyavula et al., 2013).

There is also scarce information about the change of expression levels of the key genes involved in the phenylpropanoid pathway during potato tuber development. Those studies revealed that *HCT* (hydroxycinnamoyl-CoA shikimate/quinate hydroxycinnamoyl transferase), *PAL* (phenylalanine ammonia lyase), *C4H* (cinnamate 4-hydroxylase) and *C3H* (*p*-coumarate 3-hydroxylase) showed higher expression levels in immature tubers than in mature tubers with a mild increment at the end of tuber maturity (at 110 Days After Planting (DAP)). However, the expression levels of *HQT* (hydroxycinnamoyl-CoA:quinate hydroxycinnamoyl transferase) did neither increase nor decrease during tuber development. *HQT* is the gene involved in the main pathway for the biosynthesis of chlorogenic acid in potato tubers but showed a negative correlation with the total content of phenolics during tuber development (r = -0.32). Even so, *HQT* showed higher expression levels than *PAL* and *HCT* at all developmental stages (Navarre et al., 2013). The expression of downstream genes involved in the biosynthesis of anthocyanin such as UFGT (UDP-glucose:flavonoid 3-*O*-glucosyltransferase), DFR (dihydroflavonol reductase), ANS (anthocyanin synthase), and F3H (flavanone 3-hydroxylase) tended to decrease with tuber maturation (Payyavula et al., 2013).

The regulation of ascorbic acid during potato tuber development has not received any special attention. The only study available was performed with one yellow-fleshed cultivar ('Ranger Russet'). It was found that the content of total ascorbic acid increased with tuber maturation (from non-tuberized stolons up to fully developed tubers) and decreased after harvesting (Blauer et al., 2013). They also found that the transcript levels of the genes involved in the synthesis of ascorbic acid (Smirnoff-Wheeler pathway) increased in the early stages of tuber formation, then decreased during tuber bulking and increased slightly at the end of tuber maturation. It was found in a study performed with carrots that the expression levels of the genes involved in the synthesis of ascorbic acid and the content of the total ascorbic acid increased at the early stages of development and decreased with the root development. The transcript levels of those genes correlated positively with the content of ascorbic acid (G.-L. Wang et al., 2015).

2.7 Effect of weather conditions on potato tuber formation and on the content of phenolic compounds and ascorbic acid

2.7.1 Effect of weather conditions on tuber formation

Potato dry matter is affected by the interception of the solar radiation that varies during plant development. Dry matter in potato is distributed between the tubers and the other parts of the plant. The higher the light use efficiency, the higher the dry matter produced (Haverkort, 2007).

Short day photoperiod is a strict requirement for some potato species (Solanum demissum, and some lines of Solanum tuberosum subsp. andigena), while some other species can produce tubers in a long day photoperiod. It was found that the anthocyanin content in potato leaves grown in short day conditions (8 h light) was 20 to 25 % lower than potato leaves grown under long day conditions (16 h light) (Martínez-García et al., 2001).

The induction of tuber formation is affected by environmental conditions mediated by phytochrome and gibberellins (GAs). Phytochrome is involved in many photoperiodic reactions. High levels of GAs inhibited tuberization, while low levels induced tuberization at the stolon apex (site of the newly formed tuber) (Hannapel, 2007).

The quality of light is also a factor influencing tuber formation. Supplementation of short red light treatments in the middle of the night reduced tuber formation. This reduction was partly reversed when tubers were exposed to far red light treatments (Batutis & Ewing, 1982). The effect of the quality of light on tuber formation and synthesis of metabolites in potato tubers is an area that needs to be better studied specially in high latitude areas since the proportion of red light in comparison to blue light is higher in Nordic countries.

Potato is susceptible to drought events. A study conducted under controlled irrigation conditions in two consecutive years showed that drought reduced tuber yields of yellow and purple-fleshed cultivars (Wegener & Jansen, 2013). Drought reduces plant growth, shortens growth cycle, reduces the number of tubers produced per plant and the size of tubers. Thus, the final tuber yield is seriously affected by drought events at emergence and tuberization of potatoes (Monneveux et al., 2014).

2.7.2 Effect of weather conditions on the synthesis of phenolic compounds in potato tubers

The effects of environmental factors on the synthesis of phenolic compounds in plants have been studied mainly in model plants such as Arabidopsis thaliana (Coffey & Jansen, 2019; Schulz et al., 2015, 2016, 2021) or in higher plant organs such as leaves (Harb et al., 2015; Kaling et al., 2015; Neugart et al., 2012) or fruits (Åkerström et al., 2010; Carbone et al., 2009; W. Chen et al., 2019; Del-Castillo-Alonso et al., 2016; Henry-Kirk et al., 2018; Karppinen et al., 2016; Mikulic-Petkovsek et al., 2015; Tapia et al., 2022; B. Yang et al., 2013).

In the case of potato tubers, most studies have been conducted under controlled conditions or have been performed in a specific stage of tuber development (e.g., fully mature tubers). In some cases, only one potato cultivar has been studied. Thus, information about how weather conditions affect the synthesis of phenolic compounds during the development of tubers is scarce.

In a study performed under controlled temperature conditions it was found that the phelloderm of potato tubers exposed to heat stress had down-regulated expression of the genes of the phenylpropanoid pathway. This correlated with the reduced levels of anthocyanins in the skin of the potato tubers, and a reduced skin color of the heated tubers. (Fogelman et al., 2019).

A study (performed in two consecutive years) compared the anthocyanin content in purple-fleshed potato cultivars that received daily water and cultivars that were exposed to periods of drought for six days. This study reported that exposure to drought did not significantly affect the anthocyanin content, but the year of cultivation did significantly affect the anthocyanin content. In the first year the weather was warm and sunny, so they applied a drought period of 6 days, while in the second year the weather was cool and cloudy, and therefore they applied two drought periods. The anthocyanin content was higher in the potato tubers grown in the second year than in the first year. The authors concluded that cooler temperatures in July in the second year were the determining factor that favored the production of anthocyanins (Wegener & Jansen, 2013).

Field experiments showed that potato cultivars grown at low temperatures, high solar radiation and long photoperiod produced tubers with higher content of anthocyanins than the same cultivars grown in areas exposed to opposite weather conditions (Reyes et al., 2004). Similarly, in another field study it was evaluated how latitude affects the fully matured tubers of the purple-fleshed cultivar ‘Magic Molly’ grown in the USA. It was found that potato tubers grown at higher latitudes (Alaska, 58° N to 67° N) had higher contents of total phenolic acids and anthocyanins than the tubers grown at lower latitudes such as Texas and Florida located at 33° N and 29° N, respectively. Correspondingly, the expression levels of phenylalanine ammonia-lyase (*PAL*), cinnamate 4-hydroxylase (*C4H*), hydroxycinnamoyl CoA shikimate hydroxycinnamoyl transferase (*HCT*), p-coumarate 3-hydroxylase (*C3H*), anthocyanidin synthase (*ANS*), and UDP glucose:flavonolglucosyl transferase (*UFGT*) were higher in tubers grown at northern latitudes. Interestingly, the authors found that the contents of 3-*O*-caffeoylquinic acid and 4-*O*-caffeoylquinic acid were higher in the tubers grown in Texas and Florida. Again, the content of 5-*O*-caffeoylquinic acid (the main phenolic acid in potato representing around 90 % of the total phenolic acids) was higher in the tubers harvested in Alaska (Payyavula et al., 2012).

2.7.3 Effect of weather conditions on the synthesis of ascorbic acid in potato tubers

The effect of weather conditions on the synthesis of ascorbic acid has been evaluated mainly in model plants and to a lesser extent in potato tubers.

Ascorbic acid is subject to diurnal changes affected by the circadian rhythm. The synthesis of ascorbic acid in spinach leaves is the highest at dawn before midday (Kiyota et al., 2006).

A field study was performed on thirteen potato cultivars with different flesh color grown at two locations in the Czech Republic in two consecutive years. It was found that the content of ascorbic acid in the fully matured tubers was higher when the potatoes were grown in warmer and drier conditions (16 °C and 363 mm, respectively) than when grown in cooler and wetter conditions (14.7 °C and 443 mm, respectively) (Hamouz et al., 2018).

A study conducted with 25 potato cultivars and grown at three locations in the Andes of Peru indicated that the content of ascorbic acid was in general higher at the lower altitude area (3280 masl) than at the higher altitudes (3700–3800 masl). The authors attribute the differences in the ascorbic acid content in the tubers to the different agronomical practices and to the higher soil pH of the area (7.6) than the higher altitude areas (3.6–5.2). They did not report the weather conditions of those areas (Burgos et al., 2009). However, the lower altitude areas are generally characterized by warmer conditions than the higher altitude areas.

3 AIMS OF THE STUDY

The main aim of the study was to understand the effect of weather and climatic conditions (affected by latitude and year) in Finland on the expression levels of the genes involved in the phenylpropanoid pathway and on the content of anthocyanins, phenolic acids and ascorbic acid in potato tubers collected at different stages of tuber development.

Thermal treatment degrades anthocyanins and ascorbic acid. However, some studies have failed to show that in potato tubers. Hence, the first step was to improve the extraction methods of phenolic compounds and ascorbic acid to determine accurately their contents. Our first hypothesis was that raw potatoes will have higher content of anthocyanins and ascorbic acid than cooked potatoes.

Fruits growing under low temperature conditions produce higher content of phenolic compounds and ascorbic acid. Thus, our second hypothesis was that potato plants growing at 13 °C will produce tubers with higher content of these compounds than when growing at 18 °C.

Likewise, our third hypothesis was that if we cultivate potato plants at different latitudes, potato plants growing at higher latitudes (characterized by cooler weather conditions than lower latitudes) will produce tubers with higher content of phenolic compounds and ascorbic acid than tubers produced at lower latitudes.

Overall, the objectives of the individual studies were to:

I. Improve the extraction efficiency and minimize the effect of external factors in the determination of anthocyanins and ascorbic acid in raw and cooked blue-purple potatoes.

II. Investigate how cultivar, temperature and stage of tuber development affect the accumulation of anthocyanins, phenolic acids, ascorbic acid and the expression of genes involved in the biosynthesis of phenolic compounds with emphasis on the anthocyanins in potato cultivars grown in a greenhouse under control temperature conditions.

III. Investigate how cultivar, stage of tuber development and weather conditions (affected by latitude and year) impact on the accumulation of anthocyanins, phenolic acids, ascorbic acid and the expression of genes involved in the biosynthesis of phenolic compounds in potato cultivars grown on open fields.

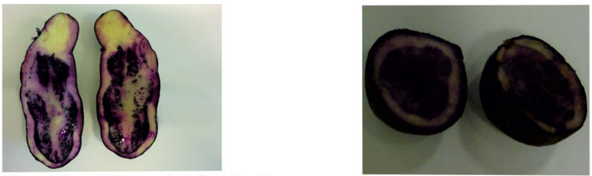
4 MATERIALS AND METHODS

4.1 Potato cultivars

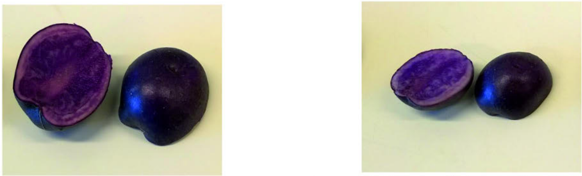
In **Study I**, five potato cultivars were investigated: two purple cultivars purchased from a market in Peru: 'Lomito Negro' and a purple cultivar from Cuzco-Peru, two purple cultivars harvested in Finland - Muhos in 2014: 'Synkeä Sakari' and 'Blue Congo', and one Chinese purple cultivar: 'Black Beauty', grown in a greenhouse in Finland in 2014.

In **Study II**, four purple cultivars ('Blue Congo', 'Blaue Veltlin', 'Blaue Schweden', and 'Synkeä Sakari'), and one yellow cultivar ('Van Gogh') were studied.

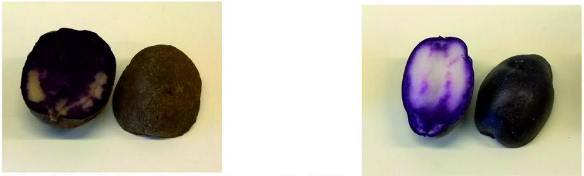
In **Study III**, four cultivars were examined: 'Blue Congo', 'Synkeä Sakari', 'Rosamunda' and 'Van Gogh'. All the cultivars studied are shown in (**Fig. 8**).



'Lomito Negro' (Study I) 'Black Beauty' (Study I)



'Blue Congo' (Study I, II, III) 'Blaue Schweden' (Study II)



'Synkeä Sakari' (Study I, II, III) 'Blaue Veltlin' (Study II)

'Rosamunda' (Study III) 'Van Gogh' (Study II, III)

Fig. 8 Potato cultivars used in **Study I**, **II** and **III**.

4.2 Experimental design

4.2.1 Method development (Study I)

Raw and steamed potato tubers with and without freeze-drying were used to quantify anthocyanins and ascorbic acid.

4.2.1.1 Method development for the quantification of anthocyanins

To improve the extraction of anthocyanins, three extraction solvents were applied: a) acidified methanol, b) acidified 50 % aqueous methanol and c) acidified 70 % aqueous methanol. All the extraction solvents contained 0.122 M HCl.

Two different amounts of samples were used (2 ± 0.001 g of frozen ground sample or 0.2 ± 0.005 g of freeze-dried powdered sample).

Recovery tests were performed with 0.2 ± 0.005 g of freeze-dried sample. The samples were spiked with 100 µL and 300 µL of the stock solution of cyanidin-3-*O*-glucoside chloride (0.1 mg/mL). Additionally, recovery studies using black currant juice concentrate were performed. Black currant juice was added at two different levels (40 or 80 µL) which were weighed for quantitative purposes.

4.2.1.2 Method development for the quantification of ascorbic acid

Freshly prepared 3% metaphosphoric acid (MPA) in water containing 1 mM/L ethylenediaminetetraacetic acid (EDTA) was used as the extraction solvent. The solvent was refrigerated at 10 °C for one hour before starting the extraction. Three different concentrations of freshly prepared water solutions of TCEP and DTT were used to reduce dehydroascorbic acid (10 mM, 100 mM and 200 mM).

For recovery purposes, the original content of ascorbic acid in the sample was determined prior to spiking. Then, 0.5 ± 0.001 g of freeze-dried powdered potato samples (raw and steamed) were spiked with 200 µL of different ascorbic acid solutions (0.40 mg AA/mL, 0.80 mg AA/mL, and 1.15 mg AA/mL).

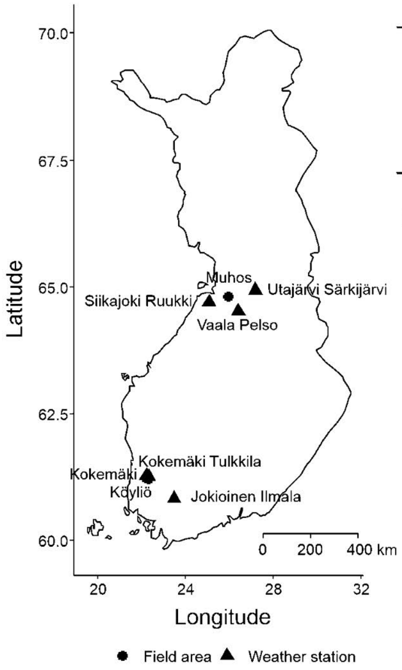
4.2.2 Greenhouse experiment (Study II)

Potato plants were cultivated in pots at two different controlled temperatures in separate rooms: 13 °C (day/night) and 18 °C (day/ night). The cultivation was performed from late January until May in the research greenhouses of the Botanical Garden of the University of Turku located in Ruissalo, Turku, Finland. Light was natural daylight, supplemented with high-pressure sodium lamps and the photoperiod was set to be 16 h (light intensity 200–500 µmol m^{-2} s^{-1}). The seed tubers were planted in pots with a capacity of three liters. The soil contained a mixture of four parts of peat and one part of sand. The plants were fertilized with a 1% N-P-K 13-7-20 (Puutarhan Kesä, Berner Oy, Helsinki, Finland) fertilizer solution in water. Watering and fertilization were performed once a week.

4.2.3 Field experiment (Study III)

Four potato cultivars were grown on the fields at two different latitudes (61.2 and 64.8° N) and in two different years (2014 and 2015) in Finland. There were in total four field trials: a) 61.2° N in 2014 (Kokemäki), b) 61.2° N in 2015 (Köyliö), c) 64.8° N in 2014 (Muhos), and d) 64.8° N in 2015 (Muhos) (**Fig. 9**).

The study included two potato cultivars with purple flesh and purple skin (‘Blue Congo’ and ‘Synkeä Sakari’), a cultivar with yellow flesh and red skin (‘Rosamunda’) and a cultivar with yellow flesh and yellow skin (‘Van Gogh’).



	Place	Latitude	Longitude
Weather station	Kokemäki Tulkkila	61.25	22.35
	Jokioinen Ilmala	60.81	23.50
	Utajärvi Särkijärvi	64.93	27.19
	Vaala Pelso	64.50	26.42
	Siikajoki Ruukki	64.68	25.09
Field area	Kokemäki	61.21	22.30
	Köyliö	61.23	22.18
	Muhos	64.82	25.95

Fig. 9 Location of potato field trials and weather stations that provided the meteorological data (**Study III**).

4.3 Sample collection

Fully mature tubers were used for **Study I**. For **Study II** and **Study III**, the collection of tubers started at 60 days after planting (DAP) and continued every 10 days up to 120 DAP. At each time point, all daughter tubers were collected from one plant per cultivar (which represented one individual plant replicate).

Study II had six individual plant replicates and **Study III** four individual plant replicates.

4.4 Sample pre-preparation

Study II and **III** were performed using freeze-dried potato tubers. Four to six tubers of each sample were divided in four parts, immersed in liquid nitrogen and send to the freeze-drier for 4 to 5 days. The dry samples were ground with a mortar and pestle with the addition of liquid nitrogen. Finally, the samples were stored at – 80 °C.

4.5 Analysis of anthocyanins and phenolic acids by HPLC-DAD and UHPLC-MS-Q-TOF

In **Study I**, total anthocyanins were quantified by reversed phase liquid chromatography coupled with diode array detector. The eluents used for this study were A: 5 % formic acid in water and B acetonitrile. Anthocyanins were detected at 520 nm and quantified as cyanidin 3-glucoside chloride.

For **Study II** and **III** anthocyanins and phenolic acids were extracted with acidified 70 % aqueous methanol. The quantification of these compounds was performed by HPLC-DAD with eluent A consisting of H_2O/acetonitrile/formic acid (87/3/10) (v/v/v), and eluent B H_2O/acetonitrile/ formic acid (40/50/10) (v/v/v). Anthocyanins were detected at 520 nm and phenolic acids at 320 nm. Quantification was performed using external calibration standards. Cyanidin 3-glucoside chloride was used for the quantification of anthocyanins and chlorogenic acid for the quantification of phenolic acids. Anthocyanins were quantified as cyanidin 3-glucoside using a correction factor for the chloride ion.

The identification of anthocyanins (**Study II and III**) was performed using UHPLC-DAD-ESI-MS, reference compounds and literature data. The analyses were performed in positive ion mode. The identification of phenolic acids (**Study III**) was performed using UHPLC-DAD-Q-TOF, as well as reference compounds and literature data. The analyses were performed in negative ion mode.

4.6 Analysis of ascorbic acid by UHPLC-DAD

For **Study II** and **III,** 0.5 ± 0.005 g of ascorbic acid was extracted with 3% MPA and 1 mM/L EDTA in water. Tris(2-carboxyethyl)phosphine hydrochloride (TCEP) was added to the extracts to reduce dehydroascorbic acid (DHA) and obtain the total amount of ascorbic acid. The reduction was carried out in dark at room temperature for one hour. Ascorbic acid was quantified using L-ascorbic acid as external standard. All the extracts were analyzed by UHPLC-DAD in the same day of preparation.

4.7 Environmental growth conditions

Meteorological data collected from the Finnish Meteorological Institute from the weather stations at Kokemäki Tulkkila, Jokioinen Ilmala, Utajärvi Särkijärvi, Vaala Pelso and Siikajoki Ruuki were used in **Study III.** Based on this, the effect of weather conditions affected by latitude and year on the potato cultivars grown in Muhos, Kokemäki and Köyliö in 2014 and 2015 were evaluated. Exact location of weather stations is shown in **Fig. 9**.

For each field trial, daily weather data were collected during the growing season (May to August).

4.8 Statistical analysis

In **Study I**, Analysis of variance (ANOVA, Two-factor with replication) was used to evaluate the effect of different extraction solvents and reducing agents; and t-test was used to compare the results from raw and steam samples. The significance level was set to be $p < 0.05$.

Statistical analyses in **Study II** and **Study III** were performed with R version 3.5.2. The effect of temperature (**Study II**) or latitude-year (**Study III**) and the effect of the developmental stage of the tubers on the content of phenolic compounds and ascorbic acid were evaluated using three-way unbalanced ANOVA. The effect of cultivar, and its interaction with temperature and stage of development was also evaluated with three-way unbalanced ANOVA. The normality of the distribution of the data for validation of the ANOVA results was performed by Normal Q-Q plots, Levene’s Test, and Shapiro-Wilk normality test on the residuals. When the data showed positively skewed distribution, log-transformation was used to make data to conform normality. Dunnett Tukey Kramer pairwise multiple comparison test adjusted for unequal variances and unequal sample sizes was used to identify the significant differences in the total amounts of anthocyanins, phenolic acids and ascorbic acid present in tubers collected at different stages of maturity in **Study II**. Pearson correlation was performed to see the relationship between the individual metabolites and the gene expression levels. Network correlation graphics were performed with the package igraph using R software (Study II).

Time series modelling using spline regression and Generalized Additive Models (GAM) was used in **Study III** to obtain predictive splines and compare the significance difference between growing locations and years.

ANOVA-simultaneous component analysis (ASCA) was used in order to evaluate the effect of cultivar and latitude-year on the gene expression levels, anthocyanin profile and phenolic acid profile in potato tubers harvested at different stages of tuber development in **Study III**.

5 RESULTS AND DISCUSSION

5.1 Method development for the determination of anthocyanins and ascorbic acid

5.1.1 Extraction and quantification of anthocyanins (Study I)

The cultivar ‘Lomito Negro’ was used in the initial stage of method development. The methods of anthocyanin isolation in the non-freeze dried and freeze-dried raw and steam samples were compared using three different extraction solvents (50 %, 70 % and 100 % acidified methanol). **Fig. 10** shows the cumulative extraction yields of six consecutive extractions in the potato samples. The samples without freeze-drying produced lower extraction yields than the freeze-dried (FD) samples (**Fig. 10a; b**). Extraction of anthocyanins in freeze-dried potatoes using 50 %, 70 % or 100 % acidified methanol produced similar extraction yields except for steamed freeze-dried samples extracted with 100 % methanol which extracted only 30 % of the anthocyanins (**Fig. 10b**). The low extraction in steamed freeze-dried samples extracted with 100 % methanol should be further investigated but might be caused by diverse factors: a) formation of stronger starch granules produced by gelatinization (after steaming) and retrogradation (producing strong intermolecular hydrogen bonding between the amylose and amylopectin molecules) (Tako et al., 2014); b) higher irregular cell arrangement and shrinkages of the cell walls in steamed freeze-dried potato than in non-steamed freeze-dried potato (R. Wang et al., 2010); and c) loss of water after freeze-drying which could enhance solubilization of anthocyanins (our freeze-dried samples had between 98 and 100 % dry matter). It was found that the addition of water (up to 25 %) to an ionic liquid caused a partial swelling of starch granules favoring the diffusion of the ionic liquid and the solubilization of the starch granules (W. Liu & Budtova, 2013). Thus, one or the sum of all these factors might have prevented the swelling of the cell walls and extraction of anthocyanins.

Like our study, another research group showed that the yield of anthocyanins in steamed freeze-dried potatoes decreased when extracted with 100 % methanol but increased when extracted with 60 % methanol (Burgos et al., 2013). The results also showed that the non-freeze-dried steamed samples (S) produced higher extraction yields than the non-freeze-dried raw samples (R) (**Fig. 10a**). Thus, steaming potato samples prior to anthocyanin extraction is an inexpensive method to achieve around 80 % recovery of the anthocyanins present in the samples. A previous study also showed that raw samples had lower anthocyanin amounts than steamed samples (Lachman et al., 2012). Additionally, slices of purple-fleshed sweet potatoes without heat treatment showed 30 % less anthocyanins than the heat-treated slices (90 °C for 10 min). Heating inactivated anthocyanases (glycosidases, phenol oxidases and phenolases) which degrade anthocyanins to anthocyanindins that later are oxidized by polyphenol oxidases (de Aguiar Cipriano et al., 2015; Enaru et al., 2021).

To continue with the method development, anthocyanin extraction was performed in raw freeze-dried and steamed freeze-dried, purple-fleshed potato cultivars. The anthocyanin yield was compared using three extractions with 70 or 50 % acidified methanol. Raw freeze-dried samples produced higher extraction yields than steamed freeze-dried samples (**Fig. 11a**). Extraction with 70 % acidified methanol was more effective than extraction with 50 % acidified methanol in the raw freeze-dried samples, whereas there were no significant differences in the extraction yield in steamed freeze-dried samples. Additionally, anthocyanin extraction yield was compared using three and four extractions with 70 % acidified methanol. ANOVA results showed that there were no significant differences between four and three extractions. Further analysis using four extractions and 70 % acidified aqueous methanol were performed with two more purple-fleshed cultivars (**Fig. 11b**). This confirmed that steaming decreased the content of anthocyanins by 15 %. Raw freeze-dried samples gave higher anthocyanin yields. Freeze-drying reduces enzyme activity and, in some cases, causes irreversible inactivation of enzymes due to freezing and drying stress that alters the enzyme structure (Carpenter et al., 1993).

To estimate the recovery rate of anthocyanins in freeze-dried samples, different amounts of cyanidin-3-*O*-glucoside or blackcurrant juice were added to freeze-dried samples (raw and steamed) of the yellow-fleshed potato cultivar ‘Rikea’. The anthocyanin recovery was 105 % when 10 µg of the cyanidin-3-*O*-glucoside standard was spiked to 200 µg of raw freeze-dried potato and 100 % when 30 µg of the standard was added. When blackcurrant juice was spiked to raw and steamed freeze-dried samples, the anthocyanin recovery was 80 % when 40 µL of juice was added and 96 % when 80 µL of juice was added.

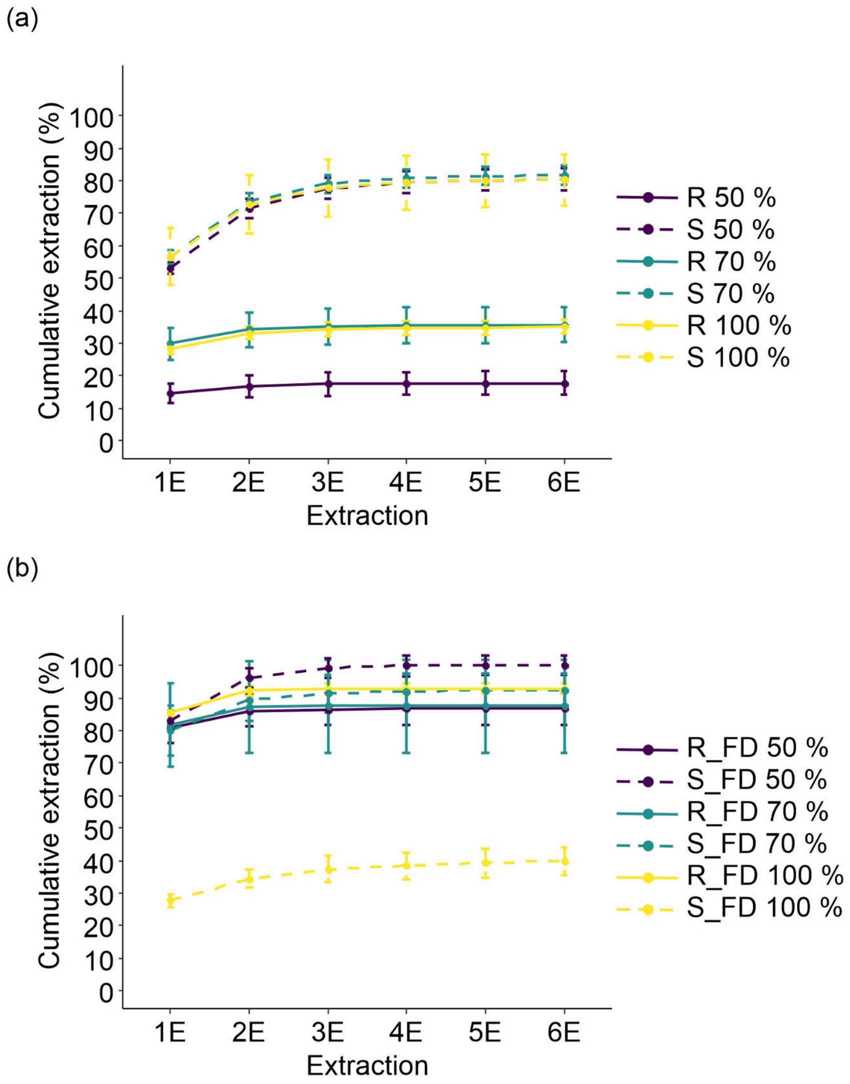


Fig. 10 Cumulative extraction yield of anthocyanins from the potato tubers of the cultivar 'Lomito Negro' using different extraction solvents in: (a) Non-freeze-dried samples, and (b) Freeze-dried samples. R = Raw, S = Steamed, FD = Freeze-dried, 50 % = acidified 50 % aqueous methanol, 70 % = acidified 70 % aqueous methanol, 100 % = acidified 100 % methanol. Anthocyanin extraction using six consecutive extractions on steam freeze-dried potato samples with 50% acidified aqueous methanol (S_FD 50%) resulted in the highest anthocyanin recovery or extraction, so six extractions with this solvent were set to be 100 % extraction yield (corresponding to the maximum total area/ g freeze-dried sample). Error bars show standard deviation among the replicates. (n = 3 in most of the samples, except in S_FD 100 % where n= 6). Modified from the original publication **I** with permission from Elsevier.

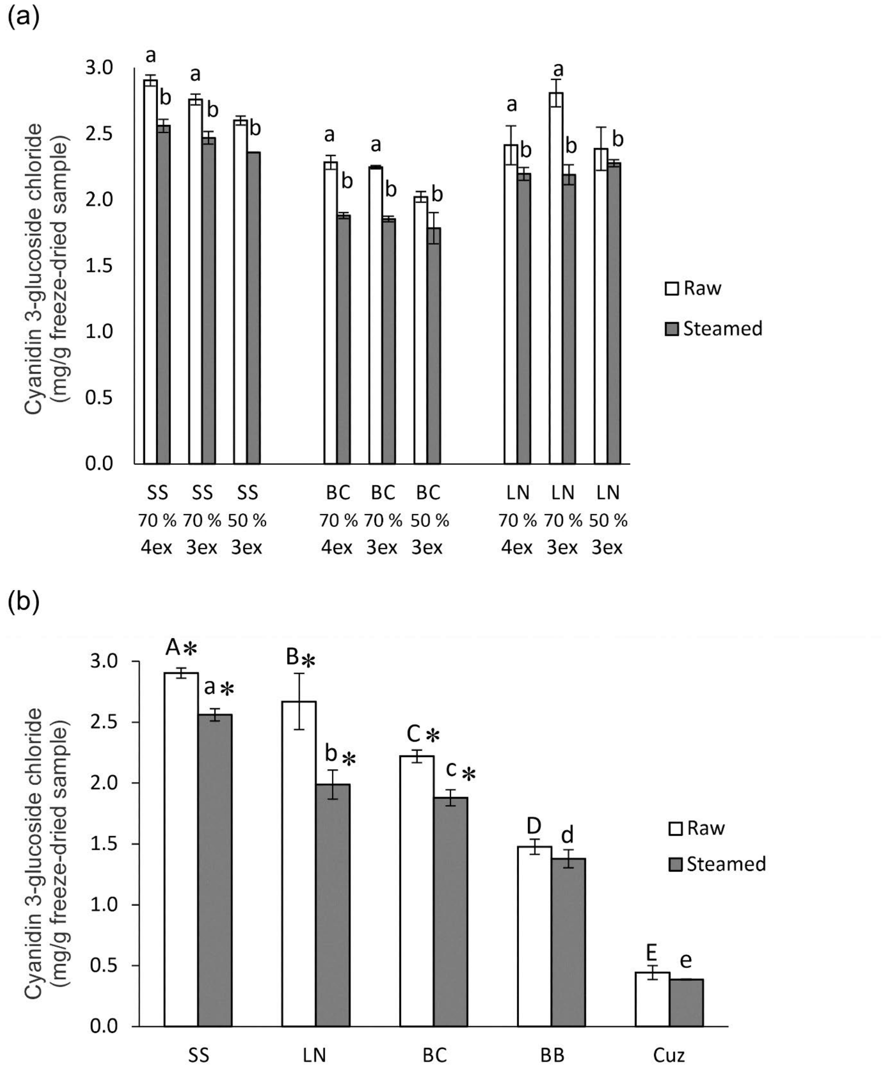


Fig. 11 Content of anthocyanins in raw and steamed freeze-dried samples. (a) Samples extracted three or four times with 50 or 70 % aqueous methanol. (b) Samples extracted four times with 70 % aqueous methanol. Anthocyanins are expressed as cyanidin-3-glucoside chloride equivalents (mg/g freeze-dried sample). SS = ‘Synkeä Sakari’; BC = ‘Blue Congo’; LN = ‘Lomito Negro’; BB = ‘Black Beauty’; Cuz = ‘Lomito Negro’ from Cuzco. Reprinted from the original publication **I** with permission from Elsevier.

5.1.2 Identification of anthocyanins (Study II, Study III)

Identification of anthocyanins was carried out for **Study II** and **Study III** in order to understand the impact of cultivar, and weather conditions (affected by latitude and year) on the anthocyanin profile, and to perform anthocyanin - anthocyanin and gene-anthocyanin correlations at later stages of the study. Anthocyanins were identified based on their UV-vis absorption, retention time, mass spectra and MS-MS data from previous published studies (Burmeister et al., 2011; Giusti et al., 2014; Hillebrand et al., 2008; Nems et al., 2015; Oertel et al., 2017).

Eighteen anthocyanins were tentatively identified in the purple-fleshed potatoes, while only two to four anthocyanins were tentatively identified in the red-skinned cultivar.

UV-visible spectral information allows us to classify the anthocyanins in various groups. All delphinidin derivatives (delphinidin, petunidin, and malvidin) showed a maximum absorbance in the range of 521 to 534 nm, while cyanidin derivatives (cyanidin and peonidin) showed a maximum absorbance from 520 to 526 nm, and pelargonidin derivatives at 508 nm. Additionally, anthocyanins acylated with organic acids showed a shoulder with an absorption in the range of 279 to 328 nm. Previous studies have reported similar information about the structural properties of anthocyanins (Hong & Wrolstad, 1990; Rodriguez-Saona et al., 1998).

The main anthocyanin in ‘Blue Congo’, ‘Blaue Schweden’ and ‘Synkeä Sakari’ was petunidin-3-*p*-coumaroylrutinoside-5-glucoside (A10, **Fig. 12a**, **Table 1**) which represented 82, 80, and 65 % of the anthocyanin fraction, respectively. MS data for this compound showed the product ion *m/z* 771 corresponding to the loss of glucose from the precursor ion (*m/z* 934.1). Thus, the ion *m/z* 771 showed that petunidin was linked to the disaccharide rutinose and *p*-coumaric acid, most likely at position 3. The precursor ion *m/z* 479.6 was a common ion found in all petunidin derivatives (A1, A6, A7a, A8, A12, A17b). This ion (*m/z* 479.6) confirms the presence of glucose linked to petunidin, probably at position 5.

The main anthocyanin in ‘Blaue Veltlin’ was malvidin-3-*p*-coumaroylrutinoside-5-glucoside (A15, **Fig. 12a**, **Table 1**) which represented 58 % of the anthocyanin fraction. The precursor ion *m/z* 948 and product ion *m/z* 331.4 confirms the malvidin derivative, while the product ion *m/z* 785.8 shows malvidin linked to rutinose and *p*-coumaric acid, and the ion *m/z* 493.5 shows malvidin linked to glucose which is also found in compounds A4 and A17a.

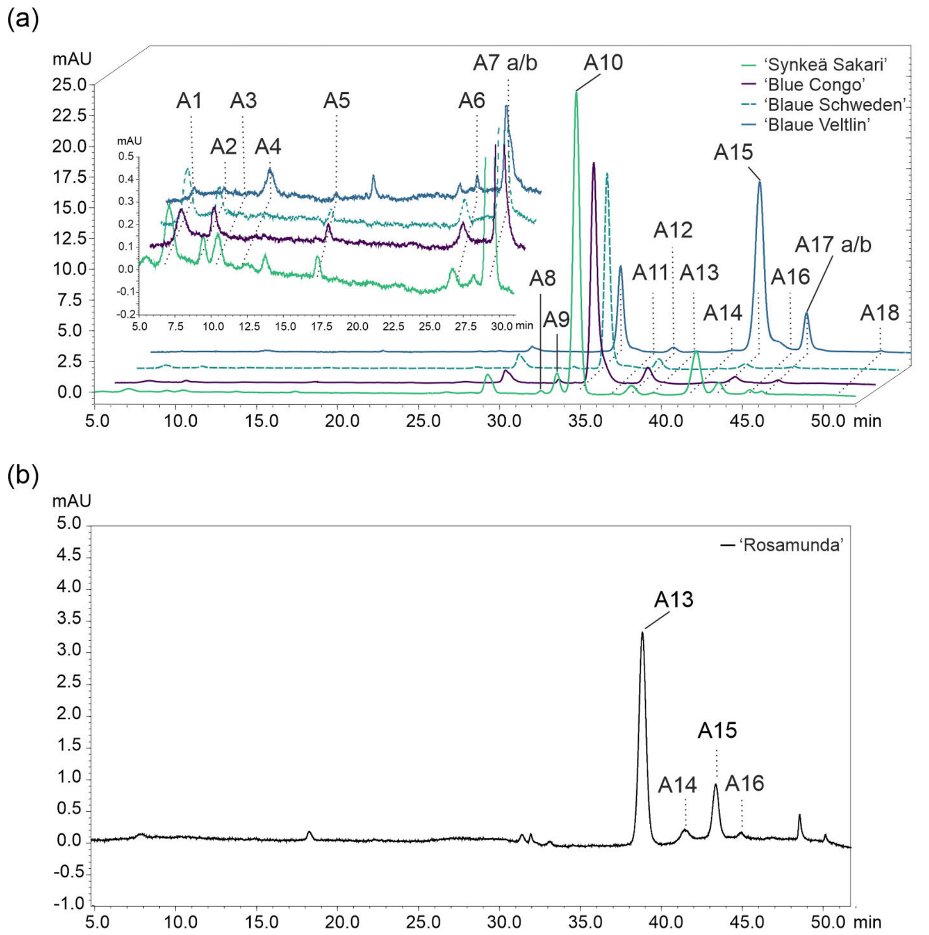


Fig. 12 HPLC-DAD chromatograms at 520 nm of: (a) Purple-fleshed potato cultivars (reprinted from the original publication **II** with permission from Elsevier); and (b) Red-fleshed potato cultivar (**Study III**).

Anthocyanins in the purple-fleshed tubers were mainly linked to the disaccharide rutinose (at position 3) and to the monosaccharide glucose (at position 5). A previous study with NMR spectroscopy demonstrated the glycosylation of potato anthocyanins with rutinose at position 5 and with glucose at position 3 (Hillebrand et al., 2008). The most common acyl moiety in the purple-fleshed potato tubers was *p*-coumaric acid, followed by ferulic acid and, to a lesser extent, caffeic acid.

In the red-fleshed potato cultivar ('Rosamunda'), the main anthocyanin was pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside (A13, **Fig. 12b**, **Table 1**) and represented 77 % of the anthocyanin fraction. The product ion *m/z* 271.3 shows the pelargonidin derivative, the product ion *m/z* 433.4 shows the pelargonidin linked to glucose, and the product ion *m/z* 725.7 shows pelargonidin linked to rutinose and *p*-coumaric acid after losing a glucose from the precursor ion *m/z* 887.8.

Table 1 Anthocyanins identified in purple-fleshed and red-skinned potato cultivars by LC-DAD-ESI-MS. Modified from the original publication **II** with permission from Elsevier.

Peak	**Ret. Time (min.)**	**UV-Vis absorption maxima (nm)**		**[M]+ (m/z)**	**Product ions (m/z)**			**Compound**	**Ref.**
A1	7.1	525	325	788.0	317.4	479.5	625.6	Petunidin-3-rutinoside-5-glucoside	d, e
A2	9.5	521	322	531.8	317.4	479.7		Petunidin derivative [a]	
A3	12.2	525	280	n.d.	303.4			Delphinidin derivative [a]	
A4	10.5	528		655.6	331.5	493.5		Malvidin derivative [a]	
A5	17.3	525		n.d.	317.4	454.0		Petunidin derivative [a]	
A6	26.7	526	279	966.0	317.5	479.5	803.8	Petunidin derivative [a]	
A7a	29.2	532	297	949.8	317.4	479.6	787.7	Petunidin-3-caffeoylrutinoside-5-glucoside	c, d, f
A7b	29.2	532	297	920.1	303.4	465.5	757.7	Delphinidin-3-*p*-coumaroylrutinoside-5-glucoside	f
A8	32.5	528	300	934.0	317.5	479.4	772.4	Petunidin *p*-coumaroylrutinoside glucoside [a]	
A9	33.5	523	300	903.9	287.4	449.6	741.8	Cyanidin-3-*p*-coumaroylrutinoside-5-glucoside	f
A10	34.7	530	301	934.1	317.5	479.6	771.9	Petunidin-3-*p*-coumaroylrutinoside-5-glucoside	c, d, e, f
A11	37.0	526	281	917.9	301.3	463.4	755.6	Peonidin *p*-coumaroylrutinoside glucoside [a]	
A12	38.2	535	324	964.0	317.3	479.5	802.5	Petunidin-3-feruloylrutinoside-5-glucoside	c, d, e, f
A13	39.5	502	314	887.8	271.3	433.4	725.7	Pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside	b, c, d, e, f
A14	42.1	520		917.9	301.3	463.4	755.7	Peonidin-3-*p*-coumaroylrutinoside-5-glucoside	b, c, d, f
A15	43.5	532	302	948.0	331.4	493.5	785.8	Malvidin-3-*p*-coumaroylrutinoside-5-glucoside	b, c, d, f
A16	45.4	513	290	947.8	301.3	463.5	785.9	Peonidin-3-feruloylrutinoside-5-glucoside	c, d, f
A17a	46.2	531	328	978.0	331.3	493.4	815.6	Malvidin-3-feruloylrutinoside-5-glucoside	d, f
A17b	46.2	531	328	961.8	317.4	479.7	771.7	Petunidin-3-*p*-coumaroylrutinoside	f
A18	50.1	533		785.9	331.5			Malvidin derivative [a]	

[a] Tentative identification by LC-DAD-ESI-MS

[b] (Burmeister et al., 2011)

[c] (Giusti et al., 2014)

[d] (Hillebrand et al., 2008)

[e] (Nems et al., 2015)

[f] (Oertel et al., 2017)

5.1.3 Identification of phenolic acids (Study II, Study III)

Although this study focused more on the analysis of anthocyanins, the determination of phenolic acids in Study II and Study III was performed by measuring the absorption at 320 nm. Analysis of the main phenolic acids in potatoes enables studying the possible correlations between the contents of anthocyanins and phenolic acids in the potato tubers and how the weather conditions affect the content of the main phenolic acids. Identification of phenolic acids in Study II was done basically according to the UV-vis absorption and the retention time of reference compounds. In Study III, identification of phenolic acids was additionally performed with UHPLC-Q-TOF-MS in negative mode and based on information from previous studies (Clifford et al., 2003; López-Cobo et al., 2014; Narváez-Cuenca et al., 2013).

All caffeoylquinic acids showed the fragment ion m/z 191 which corresponds to quinic acid ion [M – H]-. Additionally, chlorogenic acid (5-O-caffeoylquinic acid), the main phenolic acid in potatoes (Narváez-Cuenca et al., 2013), showed maximum absorption at 326 nm in the UV spectrum and the precursor ion m/z 353 in negative ion mode (**Table 2**). Dicaffeoylquinic acid showed the precursor ion m/z 515, and the fragment corresponding to the monoacylated ion (m/z 353). In the case of feruloyl quinic acid, the measured precursor ion was m/z 367.1 and the product ion with the highest intensity was m/z 191. Thus, according to previous studies (Kuhnert et al., 2010), it was possible to identify this compound as 5-O-feruloylquinic acid and differentiate it from its isomers due to its MS spectra and the intensity of the product ions.

Mono- and dihydroxycinnamic acids linked to amino compounds such as caffeoylputrescine, bis(dihydrocaffeoyl) spermine and bis(dihydrocaffeoyl) spermidine were also identified in the potato tubers. These compounds were previously found in potatoes in other studies (Narváez-Cuenca et al., 2013; Parr et al., 2005).

Caffeic acid showed an absorption at 323 nm in the UV spectrum, the precursor ion of m/z 179 in negative mode and the product ion m/z 135, which resulted from the loss of the carboxylic group from the caffeic acid.

Besides phenolic acids, it was also possible to identify the amino acid tryptophan that has been found in tuber and skin of potato samples by other studies (López-Cobo et al., 2014).

Table 2 Identification of phenolic acids (PA) in potato tuber cultivars using UHPLC-DAD-Q-TOF (**Study III**).

	Ret. Time (min)	**UV λmax (nm)**	**Measured $[M-H]^-$ (m/z)**	**Precursor ion $[M-H]^-$ (m/z)**	**Product ions (m/z)**			**Tolerance (ppm)**	**Error (ppm)**	**Molecular formula**	**Compound**
PA1	6.8	305	249.1263	249.1245	135.0463	207.1162		10	-7.4	$C_{13}H_{18}N_2O_3$	Caffeoylputrescine
PA2	8.2	279	203.0839	203.0826	142.0670	116.0513	159.0930	10	-6.0	$C_{11}H_{12}N_2O_2$	Tryptophan
PA3	8.8	281	529.3057	529.3032	365.2580	407.2686		5	-4.6	$C_{28}H_{42}N_4O_6$	Bis(dihydrocaffeoyl) spermine
PA4	10.9	319	515.1438	515.1406	353.0905	191.0578		10	-6.2	$C_{25}H_{24}O_{12}$	Dicaffeoylquinic acid
PA5	13.5	326	353.0909	353.0878	191.0577			10	-8.9	$C_{16}H_{18}O_9$	Chlorogenic acid
PA6	15.3	323	179.0361	179.0350	135.0464			9	-6.2	$C_9H_8O_4$	Caffeic acid
PA7	15.3	282	472.2475	472.2453	308.1994	186.1621		10	-4.8	$C_{25}H_{35}N_3O_6$	Bis(dihydrocaffeoyl) spermidine
PA8	18.9	311	337.0946	337.0929	191.0574	173.0467	163.0414	10	-5.1	$C_{16}H_{18}O_8$	Coumaroyl quinic acid
PA9	21.1	323	367.1056	367.1035	191.0575	173.0468	134.0384	10	-5.8	$C_{17}H_{20}O_9$	5-*O*-Feruloylquinic acid

5.1.4 Determination of ascorbic acid (Study I)

Total ascorbic acid or Vitamin C is the sum of ascorbic acid and dehydroascorbic acid. Total ascorbic acid was determined in the samples using the subtraction method. First, ascorbic acid (AA) was determined, and then dehydroascorbic acid (DHA) was reduced to ascorbic acid by addition of a reducing agent. Finally, DHA was calculated by subtraction of AA from total ascorbic acid (TAA). The most common reducing agent is dithiothreitol (DTT). However, DTT is more stable at alkaline pH. Therefore, the reducing power of TCEP was compared with that of DTT.

Total ascorbic acid was determined in raw and steamed, non-freeze dried and freeze-dried potato samples by using different concentrations of DTT or TCEP.

First, ascorbic acid was quantified in raw and steamed, non-freeze-dried and freeze-dried samples without addition of the reducing agent (**Fig. 13**). The results showed that the steamed non-freeze-dried samples showed the highest content of ascorbic acid (AA). The use of non-freeze-dried steamed potato samples increased the ascorbic acid content from 200 to 500 % compared to the non-freeze-dried raw samples. A study had shown that broccoli samples without heat treatment or exposed to mild heat treatments (30 to 60 °C for 15 min) contained less vitamin C (mainly in the form of DHA) than samples exposed to stronger heat treatments (70 to 90 °C) where L-ascorbic acid was the main form of vitamin C. The study also showed that temperatures above 60 °C strongly inactivated ascorbic acid oxidase (Wambui Munyaka et al., 2010). Thus, although ascorbic acid is heat thermolabile (Vishwasrao & Ananthanarayan, 2018), heat treatment inactivated ascorbic acid oxidase and reduced the degradation of L-ascorbic acid to DHA, which is subsequently degraded to the unstable diketogulonic acid. Consequently, heat treatment helped to stabilize L-ascorbic acid in the potato samples.

When a reducing agent was added during the extraction process to raw non-freeze-dried samples, addition of 300 µL of 10 mM TCEP or 100 mM TCEP produced higher reduction of DHA to AA (higher total ascorbic acid yields) than 300 µL of 10 mM DTT or 100 mM DTT. When the reducing agents were added to steamed non-freeze-dried samples, the addition of DTT or TCEP at different concentrations did not show significant differences in the content of total ascorbic acid (except for 10 mM TCEP in ‘Van Gogh’). It was found that in most of the cases, the content of ascorbic acid in the steamed (non-freeze-dried and freeze-dried) samples was similar with and without addition of a reducing agent (DTT or TCEP). These results suggest that most of the ascorbic acid in potatoes is in the reduced form (L-ascorbic acid) and that DHA might be formed in the raw samples during sample preparation. This confirms the results from a previous study (Wambui Munyaka et al., 2010) where the authors found DHA as the main form of ascorbic acid in raw samples. Additionally, the use of steaming on potato samples would be a fast and inexpensive technique for the determination of ascorbic acid, when screening several potato samples, since the addition of a reducing agent did not significantly increase the extraction yield of total ascorbic acid.

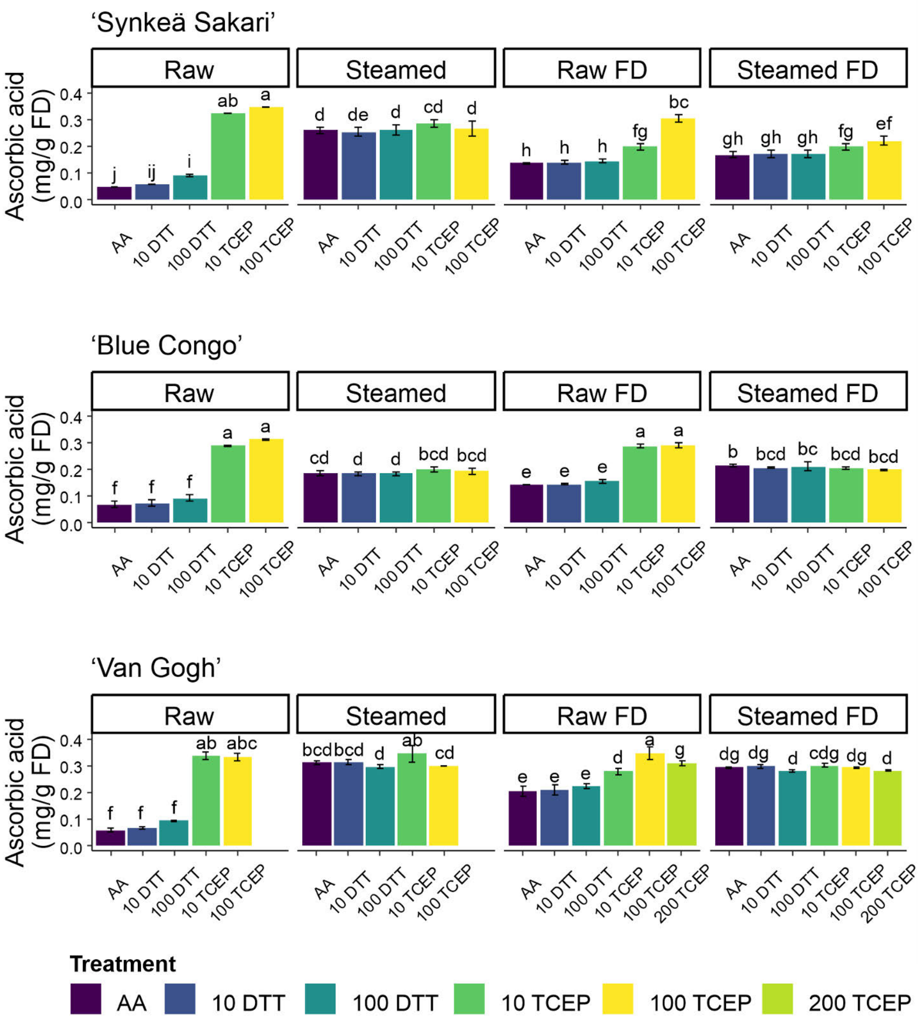


Fig. 13 Effect of different reducing agents on the final concentration of ascorbic acid in tuber samples. The extract was diluted 1:1 with H_2O or with the reducing agents at different concentrations (10 mM DTT, 100 mM DTT, 10 mM TCEP, 100 mM TCEP, or 200 mM TCEP). FD = Freeze-dried. Two-way ANOVA and Tukey test was performed for each cultivar separately. Bars labelled with the same letter are not statistically different according to Tukey test within each cultivar ($p < 0.05$) ($n = 3$). Modified from the original publication **I** with permission from Elsevier.

As a summary, addition of TCEP to raw samples produced higher reduction of DHA than DTT, and 100 mM TCEP produced higher reduction of DHA than 10 mM TCEP in raw freeze-dried samples in most of the cases (although there was an interaction effect with cultivar). This confirms the stronger reduction properties of TCEP than DTT. A previous study compared the reduction efficiency of TCEP and DTT in plasma samples and found that TCEP produced faster reduction of DHA at lower concentrations than DTT. Further, TCEP-reduced samples showed more stable concentrations of ascorbic acid than DTT-reduced samples over a 96-hour period in the autosampler at 4 °C (Lykkesfeldt, 2000).

Spike and recovery experiments were performed to further validate the method using freeze-dried samples (raw and steamed). Ascorbic acid was determined in the samples before the addition of the reducing agent and total ascorbic acid was determined after the addition of the reducing agent (100 mM TCEP). Samples were spiked with 200 µL of three different concentrations of ascorbic acid (Standard A = 0.40 mg/mL, Standard B = 0.80 mg/mL and Standard C = 1.15 mg/mL).

Addition of TCEP improved the recovery rate (between 90 and 93 %) of spiked ascorbic acid to the raw freeze-dried samples, while samples without the addition of TCEP showed low recovery rates (between 15 to 41 %). This shows that ascorbic acid is easily oxidized to dehydroascorbic acid during extraction. Thus, addition of the reducing agent enhanced reduction of DHA partly formed during extraction and converted it back to ascorbic acid. On the other hand, addition of TCEP to steamed freeze-dried samples did not show a significant impact on the recovery of ascorbic acid since the main form of ascorbic acid in steamed samples is the reduced L-ascorbic acid.

Because freeze-dried samples preserve better during storage, the selected variables for the determination of ascorbic acid were the use of raw freeze-dried samples and 100 mM TCEP as reducing agent.

Table 3 Recovery of ascorbic acid in purple potato 'Blue Congo'. Modified from the original publication **I** with permission from Elsevier.

		Raw Freeze-dried					**Steamed Freeze-dried**				
	Spiked	n	Average (µg/mL)	SD	RSD (%)	Recovery (%)	n	Average (µg/mL)	SD	RSD (%)	Recovery (%)
AA	No spiked	8	2.15	0.35	16.11		9	3.71	0.07	1.93	
Total AA	No spiked	8	4.93	0.12	2.41		9	3.74	0.11	2.87	
AA	Std. A	6	2.44	0.12	5.00	14.97	6	5.33	0.06	1.19	82.92
Total AA	Std. A	6	6.71	0.13	1.89	91.32	6	5.41	0.05	0.88	85.15
AA	Std. B	6	3.44	0.38	11.16	32.65	6	7.25	0.15	2.09	89.62
Total AA	Std. B	6	8.59	0.32	3.70	92.87	6	7.54	0.17	2.31	96.25
AA	Std. C	6	4.56	0.73	15.98	41.19	6	8.91	0.15	1.65	88.71
Total AA	Std. C	6	10.18	0.17	1.70	89.63	6	9.23	0.08	0.85	93.62

5.2 Effect of cultivar on crop yield, and synthesis of anthocyanins, phenolic acids and ascorbic acid in potato tubers (Study II and Study III)

Cultivar was the main variable affecting tuber yields as well as the synthesis of phenolic compounds and ascorbic acid. The mean content of total phenolic compounds and ascorbic acid (in **Study III)**, as well as the mean tuber yields is shown in **Table 4** and **Table 5**.

Table 4 Total content of anthocyanins, phenolic acids, ascorbic acid, number of tubers and tuber yield in the purple-fleshed cultivars (**Study III**).

Cultivar	Lat.	Year	DAP	Anthocyanins (mg/g FD sample)	Phenolic acids (mg/g FD sample)	Ascorbic acid (mg/g FD sample)	Number of tubers per plant	Yield (g/ plant)
BC	61	2014	70	1.81 ± 0.33	5.88 ± 0.30	1.09 ± 0.11	12.00 ± 4.08	207.35 ± 97.36
BC	61	2014	80	1.41 ± 0.26	4.79 ± 1.57	0.97 ± 0.08	19.75 ± 7.93	477.42 ± 84.83
BC	61	2014	89	1.11 ± 0.34	2.86 ± 0.75	0.83 ± 0.17	18.00 ± 6.88	889.63 ± 378.80
BC	61	2014	101	1.32 ± 0.21	3.82 ± 0.92	0.61 ± 0.13	32.25 ± 19.22	1179.95 ± 642.38
BC	61	2014	111	1.01 ± 0.17	2.21 ± 0.35	0.76 ± 0.13	27.75 ± 8.62	1080.40 ± 309.36
BC	61	2014	118	1.04 ± 0.04	2.74 ± 0.41	0.71 ± 0.10	18.50 ± 5.26	1032.20 ± 475.52
BC	61	2014	132	1.00 ± 0.08	1.88 ± 0.17	0.75 ± 0.40	19.00 ± 12.14	839.16 ± 368.04
BC	61	2015	60	3.37 ± 0.19	15.43 ± 1.30	1.54 ± 0.13	8.25 ± 6.75	16.62 ± 22.23
BC	61	2015	70	2.48 ± 0.79	13.69 ± 1.89	1.23 ± 0.06	16.50 ± 2.38	87.19 ± 71.67
BC	61	2015	79	2.66 ± 0.38	9.84 ± 1.37	1.51 ± 0.11	13.25 ± 2.99	178.75 ± 89.02
BC	61	2015	91	2.37 ± 0.21	8.95 ± 2.22	1.41 ± 0.09	11.50 ± 6.61	263.11 ± 113.83
BC	61	2015	100	1.65 ± 0.17	5.76 ± 0.84	1.25 ± 0.07	11.75 ± 1.71	445.89 ± 35.95
BC	61	2015	109	1.40 ± 0.17	4.27 ± 0.84	1.17 ± 0.14	13.50 ± 5.07	656.16 ± 250.20
BC	61	2015	119	1.62 ± 0.28	4.95 ± 1.60	1.06 ± 0.08	13.75 ± 7.50	671.91 ± 264.53
BC	64	2014	62	2.81 ± 0.28	8.33 ± 2.63	1.50 ± 0.09	8.00 ± 4.00	89.28 ± 73.77
BC	64	2014	70	2.62 ± 0.35	7.28 ± 1.74	1.41 ± 0.20	9.00 ± 1.63	129.90 ± 47.94
BC	64	2014	80	2.40 ± 0.36	5.18 ± 1.51	1.51 ± 0.17	10.00 ± 7.44	309.80 ± 189.93
BC	64	2014	90	1.76 ± 0.39	2.78 ± 0.63	1.29 ± 0.20	17.00 ± 11.20	730.83 ± 322.82
BC	64	2014	101	1.85 ± 0.14	3.66 ± 0.21	0.61 ± 0.36	9.75 ± 3.40	446.00 ± 225.53
BC	64	2015	60	0.54 ± 0.12	4.18 ± 1.36	0.67 ± 0.51	6.50 ± 4.04	26.30 ± 26.79
BC	64	2015	70	1.62 ± 0.48	5.47 ± 0.70	1.16 ± 0.09	11.00 ± 4.08	175.45 ± 67.30
BC	64	2015	80	1.91 ± 0.18	5.01 ± 0.32	1.29 ± 0.14	8.00 ± 4.76	344.05 ± 199.44
BC	64	2015	91	1.85 ± 0.39	3.66 ± 0.76	1.18 ± 0.10	15.00 ± 4.97	953.35 ± 208.79
BC	64	2015	99	1.91 ± 0.19	3.74 ± 1.65	0.94 ± 0.03	6.75 ± 5.44	293.93 ± 294.75
SS	61	2014	80	2.38 ± 0.63	4.34 ± 1.80	1.12 ± 0.08	16.25 ± 4.99	749.95 ± 61.67
SS	61	2014	89	1.56 ± 0.32	2.31 ± 0.41	0.91 ± 0.03	13.50 ± 3.32	768.50 ± 109.90
SS	61	2014	101	1.86 ± 0.43	2.81 ± 0.64	0.85 ± 0.07	16.25 ± 4.11	874.60 ± 199.77
SS	61	2014	111	1.89 ± 0.88	2.00 ± 0.53	0.74 ± 0.13	12.75 ± 4.99	716.52 ± 308.06
SS	61	2014	118	2.38 ± 0.30	3.13 ± 0.32	0.94 ± 0.11	17.50 ± 6.81	1062.67 ± 311.98
SS	61	2014	132	1.54 ± 0.57	1.85 ± 0.42	0.67 ± 0.11	11.00 ± 2.83	695.49 ± 177.52
SS	61	2015	60	5.28 ± 0.12	11.98 ± 1.17	1.81 ± 0.30	7.75 ± 5.32	21.11 ± 20.50
SS	61	2015	70	5.36 ± 2.25	13.07 ± 0.81	1.21 ± 0.03	23.25 ± 16.28	108.25 ± 32.76
SS	61	2015	79	3.99 ± 1.06	10.31 ± 2.06	1.67 ± 0.14	10.25 ± 4.35	179.54 ± 143.84
SS	61	2015	91	3.97 ± 0.78	8.89 ± 1.67	1.67 ± 0.29	9.00 ± 0.82	364.56 ± 264.02
SS	61	2015	100	3.82 ± 0.74	7.25 ± 1.05	1.35 ± 0.18	12.00 ± 3.65	578.18 ± 194.47
SS	61	2015	109	3.55 ± 0.51	5.47 ± 0.76	1.44 ± 0.46	10.50 ± 3.11	515.22 ± 281.87
SS	61	2015	119	3.08 ± 0.68	5.04 ± 0.69	1.15 ± 0.47	10.25 ± 3.86	561.60 ± 417.20
SS	64	2014	62	4.18 ± 0.39	4.32 ± 0.55	1.96 ± 0.09	9.50 ± 1.29	216.88 ± 88.86
SS	64	2014	70	4.87 ± 0.42	4.71 ± 0.56	2.26 ± 0.71	9.75 ± 4.35	271.30 ± 137.87
SS	64	2014	80	3.68 ± 1.10	3.23 ± 1.00	1.59 ± 0.22	10.75 ± 4.19	398.68 ± 132.56
SS	64	2014	90	3.59 ± 0.16	2.91 ± 0.61	1.54 ± 0.06	10.50 ± 3.11	547.05 ± 233.44
SS	64	2014	101	4.64 ± 0.69	3.20 ± 0.81	1.59 ± 0.25	8.75 ± 1.50	484.23 ± 60.60
SS	64	2015	60	2.71 ± 1.43	7.87 ± 0.19	0.95 ± 0.41	7.75 ± 2.06	84.83 ± 64.65
SS	64	2015	70	3.66 ± 1.47	8.48 ± 2.75	1.33 ± 0.17	11.75 ± 5.91	271.97 ± 207.51
SS	64	2015	80	2.94 ± 0.92	4.66 ± 0.89	1.48 ± 0.39	11.75 ± 3.30	443.02 ± 138.00
SS	64	2015	91	3.07 ± 1.15	4.47 ± 1.59	1.60 ± 0.21	12.50 ± 3.00	591.85 ± 72.54
SS	64	2015	99	2.54 ± 0.38	3.81 ± 0.91	1.46 ± 0.50	10.25 ± 3.77	616.90 ± 176.00

BC= ‘Blue Congo’and SS = ‘Synkeä Sakari’

Table 5 Total content of anthocyanins, phenolic acids, ascorbic acid, number of tubers and tuber yield in the cultivars (**Study III**).

Cultivar	Lat.	Year	DAP	Anthocyanins (mg/g FD sample)	Phenolic acids (mg/g FD sample)	Ascorbic acid (mg/g FD sample)	Number of tubers per plant	Yield (g/ plant)
RM	61	2014	70	0.03 ± 0.01	0.69 ± 0.18	0.90 ± 0.07	8.25 ± 3.59	240.97 ± 171.43
RM	61	2014	80	0.02 ± 0.01	0.67 ± 0.12	0.79 ± 0.06	15.25 ± 5.06	895.75 ± 200.24
RM	61	2014	89	0.01 ± 0.00	0.42 ± 0.03	0.69 ± 0.06	13.75 ± 5.85	1010.35 ± 431.97
RM	61	2014	101	0.02 ± 0.01	0.53 ± 0.20	0.54 ± 0.03	14.75 ± 3.30	1258.53 ± 444.31
RM	61	2014	111	0.01 ± 0.00	0.28 ± 0.04	0.69 ± 0.08	15.25 ± 4.99	1520.86 ± 132.57
RM	61	2014	118	0.01 ± 0.00	0.47 ± 0.04	0.69 ± 0.07	19.50 ± 7.59	1718.78 ± 439.07
RM	61	2014	132	0.01 ± 0.00	0.27 ± 0.08	0.61 ± 0.04	12.00 ± 3.37	1075.27 ± 142.69
RM	61	2015	60	0.17 ± 0.03	4.00 ± 0.44	1.79 ± 0.09	17.50 ± 4.65	56.91 ± 26.09
RM	61	2015	70	0.21 ± 0.05	3.28 ± 0.94	0.95 ± 0.12	17.75 ± 3.59	204.05 ± 116.11
RM	61	2015	79	0.19 ± 0.05	1.73 ± 0.21	1.35 ± 0.06	12.25 ± 2.87	283.19 ± 80.02
RM	61	2015	91	0.18 ± 0.03	1.57 ± 0.15	1.17 ± 0.22	10.75 ± 2.87	520.67 ± 59.11
RM	61	2015	100	0.12 ± 0.01	1.07 ± 0.08	1.05 ± 0.13	12.50 ± 4.80	698.58 ± 378.54
RM	61	2015	109	0.09 ± 0.01	0.84 ± 0.17	1.18 ± 0.03	9.25 ± 3.86	538.62 ± 119.13
RM	61	2015	119	0.08 ± 0.01	0.89 ± 0.12	1.08 ± 0.08	12.00 ± 6.22	827.65 ± 244.37
RM	64	2014	62	0.05 ± 0.03	1.14 ± 0.40	1.73 ± 0.12	11.00 ± 4.69	420.20 ± 76.44
RM	64	2014	70	0.07 ± 0.02	1.11 ± 0.24	1.41 ± 0.06	15.00 ± 10.03	334.20 ± 93.40
RM	64	2014	80	0.03 ± 0.02	0.94 ± 0.16	1.22 ± 0.18	11.25 ± 4.92	662.48 ± 189.23
RM	64	2014	90	0.05 ± 0.02	0.59 ± 0.19	0.89 ± 0.11	12.00 ± 1.41	834.85 ± 456.41
RM	64	2014	101	0.03 ± 0.01	0.64 ± 0.13	1.04 ± 0.25	13.50 ± 3.70	736.20 ± 367.22
RM	64	2015	60	0.05 ± 0.01	1.13 ± 0.13	1.10 ± 0.24	10.75 ± 4.50	158.60 ± 42.38
RM	64	2015	70	0.07 ± 0.01	0.69 ± 0.07	1.30 ± 0.07	9.25 ± 2.63	349.90 ± 162.06
RM	64	2015	80	0.08 ± 0.04	0.95 ± 0.40	1.16 ± 0.04	10.50 ± 1.91	698.17 ± 299.90
RM	64	2015	91	0.06 ± 0.02	0.52 ± 0.12	1.20 ± 0.09	9.00 ± 2.94	656.52 ± 361.43
RM	64	2015	99	0.05 ± 0.02	0.49 ± 0.12	0.96 ± 0.05	10.50 ± 1.91	895.98 ± 286.77
VG	61	2014	70	n.d.	0.75 ± 0.24	0.95 ± 0.07	13.50 ± 2.38	425.77 ± 140.74
VG	61	2014	80	n.d.	0.77 ± 0.14	0.64 ± 0.10	26.50 ± 6.61	1112.62 ± 118.17
VG	61	2014	89	n.d.	0.64 ± 0.15	0.56 ± 0.03	16.75 ± 4.65	797.45 ± 229.54
VG	61	2014	101	n.d.	0.79 ± 0.11	0.51 ± 0.04	25.25 ± 7.41	1697.17 ± 405.62
VG	61	2014	111	n.d.	0.43 ± 0.10	0.62 ± 0.08	22.50 ± 10.66	1847.67 ± 644.47
VG	61	2014	118	n.d.	0.63 ± 0.14	0.73 ± 0.10	19.75 ± 4.72	1421.53 ± 230.39
VG	61	2014	132	n.d.	0.32 ± 0.08	0.69 ± 0.16	16.50 ± 7.94	1467.09 ± 786.53
VG	61	2015	60	n.d.	6.63 ± 3.90	1.42 ± 0.08	16.25 ± 9.91	98.42 ± 69.30
VG	61	2015	70	n.d.	2.93 ± 0.75	0.84 ± 0.16	16.25 ± 5.19	314.02 ± 121.65
VG	61	2015	79	n.d.	2.26 ± 0.60	1.24 ± 0.08	20.75 ± 9.74	528.32 ± 220.01
VG	61	2015	91	n.d.	1.40 ± 0.11	1.45 ± 0.17	18.75 ± 2.75	778.59 ± 278.08
VG	61	2015	100	n.d.	0.80 ± 0.30	1.32 ± 0.08	17.00 ± 1.83	812.78 ± 269.54
VG	61	2015	109	n.d.	0.86 ± 0.31	1.32 ± 0.11	17.25 ± 5.97	782.35 ± 196.85
VG	61	2015	119	n.d.	1.25 ± 0.52	1.24 ± 0.12	21.25 ± 5.56	1214.34 ± 403.34
VG	64	2014	62	n.d.	0.86 ± 0.08	1.64 ± 0.12	24.25 ± 9.00	611.15 ± 169.11
VG	64	2014	70	n.d.	1.40 ± 0.15	1.40 ± 0.10	26.00 ± 5.89	514.23 ± 59.98
VG	64	2014	80	n.d.	0.65 ± 0.14	1.03 ± 0.17	13.50 ± 6.61	587.30 ± 313.47
VG	64	2014	90	n.d.	0.74 ± 0.18	0.96 ± 0.07	19.25 ± 12.12	1036.50 ± 504.49
VG	64	2014	101	n.d.	0.86 ± 0.04	0.87 ± 0.22	23.25 ± 7.04	777.15 ± 319.00
VG	64	2015	60	n.d.	1.44 ± 0.20	1.06 ± 0.12	17.00 ± 4.08	404.98 ± 56.18
VG	64	2015	70	n.d.	1.01 ± 0.23	1.02 ± 0.06	14.25 ± 2.36	532.52 ± 204.37
VG	64	2015	80	n.d.	0.71 ± 0.18	1.29 ± 0.09	18.25 ± 3.30	746.10 ± 73.49
VG	64	2015	91	n.d.	0.71 ± 0.36	1.06 ± 0.05	17.50 ± 5.92	951.60 ± 193.41
VG	64	2015	99	n.d.	0.37 ± 0.03	0.96 ± 0.07	15.00 ± 4.62	730.90 ± 303.04

RM = ‘Rosamunda’, VG = ‘Van Gogh’

5.2.1 Effect of cultivar on crop yield

The final tuber yield, independently from the temperature, was the highest in the cultivars ‘Blue Congo’ and ‘Van Gogh’ when cultivated under controlled conditions (Study II) in a greenhouse (**Fig. 14a**). However, when cultivated in the open field (Study III) ‘Van Gogh’ and ‘Rosamunda’ had higher tuber yields than the purple-fleshed cultivars (‘Blue Congo’ and ‘Synkeä Sakari’) at the final stage of tuber maturity (**Fig. 14b**). This was independently from the latitude and year of cultivation.

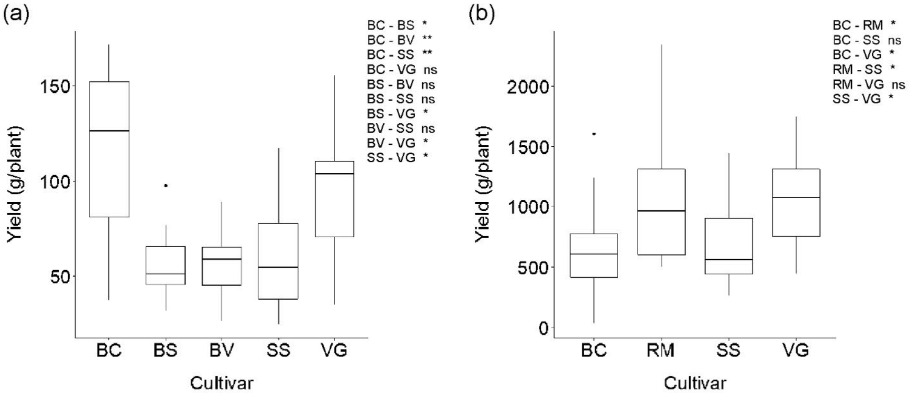


Fig. 14 Box plots showing the final yield of fully matured tubers in (a) potato tubers grown in a greenhouse in Ruissalo, and (b) potato tubers grown in the field experiments. Fully matured tubers in Ruissalo were collected at 120 DAP, and fully matured tubers in the field experiment were collected at 100 DAP and 120 DAP depending on the latitude where the samples were cultivated (**Study II**).

5.2.2 Effect of cultivar on the biosynthesis of anthocyanins

The expression levels of the genes involved in the phenylpropanoid pathway differ according to the cultivar. PC1 of the ASCA model explained 83 % of the variance (**Fig. 15a**) and separated the purple-fleshed cultivars from the yellow fleshed cultivars. Purple-fleshed cultivars (‘Blue Congo’ and ‘Synkeä Sakari’) had higher gene expression levels than the yellow-fleshed or red-skinned cultivars (‘Van Gogh’ and ‘Rosamunda’, respectively). PC2 explained 15 % of the variance and helped to show the differences between the purple-fleshed cultivars. The loadings plot (**Fig. 15b**) shows that ‘Synkeä Sakari’ had higher expression levels of *DFR* than ‘Blue Congo’. All the cultivars producing anthocyanins had high expression levels of the genes: *ANS*, *AOMT3*, *F3'5'H*, *CHS*, and *4CL* (**Fig. 15c**). The purple-fleshed cultivars had even higher *3ORT*, *F3GT*, and *F3H* expression levels than the yellow-fleshed or red-skinned cultivars. Likewise, a study showed higher expression levels of the genes involved in the synthesis of anthocyanins in a purple-fleshed potato cultivar than a white-fleshed potato cultivar (Y. Liu et al., 2015).

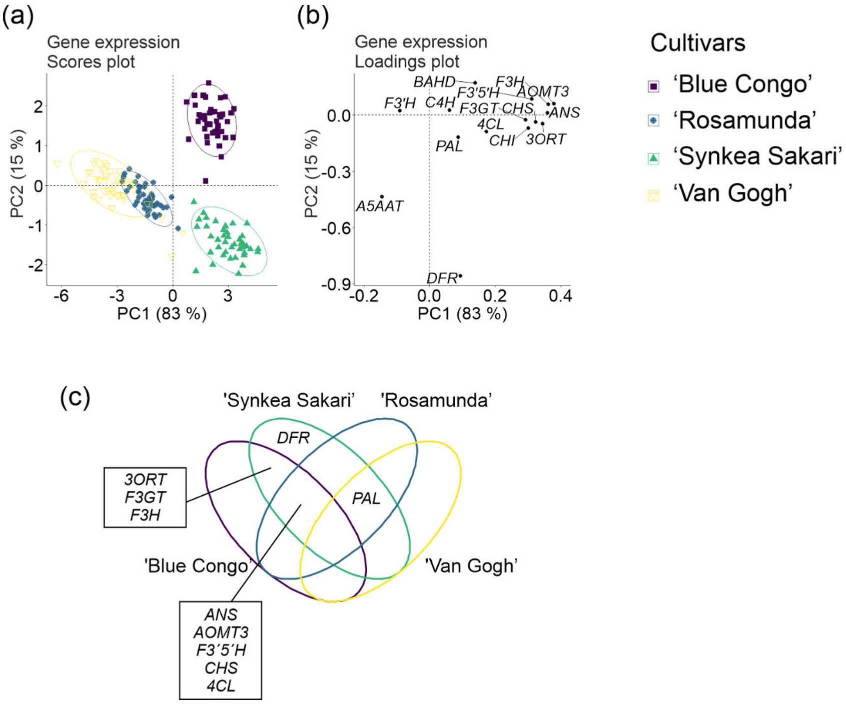


Fig. 15 Effect of cultivar on the expression levels of the genes involved in the phenylpropanoid pathway. (a) Scores and (b) Loadings plot of the ANOVA-simultaneous component analysis (ASCA) model based on the effect of "Cultivar" (c) Ven Diagram showing the genes with the highest expression levels in each cultivar (**Study III**).

The flesh of 'Synkeä Sakari' has yellow and purple areas. Thus, we studied how the gene expression levels changed according to the flesh color. The genes *CHS*, *CHI*, *F3H*, *F3'5'H*, *DFR*, *ANS*, *F3GT*, *3'ORT* and *AOMT3* were statistically significantly more induced in the purple-fleshed areas as compared to yellow-fleshed areas of the same tubers ($p < 0.05$, $n = 3$) (**Fig. 16**). Similar results were found in a previous study (Stushnoff et al., 2010) which showed that 331 genes were up-regulated in the purple areas compared to the white areas, while only eight genes were down-regulated in the purple areas.

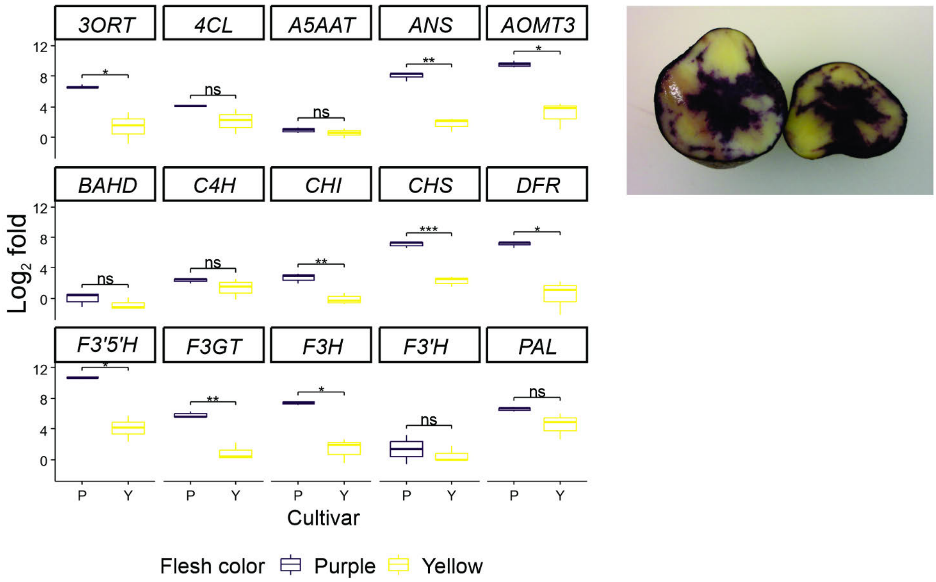


Fig. 16 Box plots showing the differential gene expression analysis between the purple and yellow flesh areas of 'Synkeä Sakari' collected in Kokemäki at 111 DAP (61 °N – 2014); p values of T test are shown in the box plots and marked with asterisks (ns = not significant). Statistically significant differences are shown with asterisks $p < 0.001$ (***), $p < 0.01$ (**), $p < 0.05$ (*) (**Study III**).

5.2.3 Effect of cultivar on the content of anthocyanins, phenolic acids and ascorbic acid

ASCA models were used to identify the differences in the anthocyanin profiles between cultivars. PC1 of the ASCA model (**Fig. 17a**) explained 53 % of the variance. The main anthocyanin causing the variance was A10 (petunidin-3-*p*-coumaroylrutinoside-5-glucoside). PC2 explained 27 % of the variance, which was mainly caused by A3 (delphinidin derivative), A9 (cyanindin-3-*p*-coumaroylrutinoside-5-glucoside), A14 (peonidin-3-*p*-coumaroylrutinoside-5-glucoside) and A15 (malvidin-3-*p*-coumaroylrutinoside-5-glucoside).

This model showed that there were no differences in the anthocyanin profiles of 'Blue Congo' and 'Blaue Schweden'. This similarity is because 'Blue Congo' is the parent genotype of 'Blaue Schweden' (Hutten & Van Berloo, 2001). However, the anthocyanin profiles of these two cultivars differed from the anthocyanin profile of 'Synkeä Sakari' and 'Blaue Veltlin'.

The main anthocyanin in 'Synkeä Sakari', 'Blue Congo' and 'Blaue Schweden' was petunidin-3-*p*-coumaroylrutinoside-5-glucoside (A10), These samples are located on the left side of the PCA plot, while samples of 'Blaue Veltlin', whose main anthocyanin was malvidin-3-*p*-coumaroylrutinoside-5-glucoside (A15), are located on the lower right side of the plot (**Fig. 17a**).

‘Synkeä Sakari’ had higher content of cyanidin-3-*p*-coumaroylrutinoside-5-glucoside (A9) and peonidin-3-*p*-coumaroylrutinoside-5-glucoside (A14) than the other pigmented cultivars. Additionally, ‘Synkeä Sakari’ had small amounts of A3, but the other cultivars did not have that anthocyanin. Most of the samples of ‘Van Gogh’ did not contain anthocyanins, thus the samples of these cultivar are located on the upper right side of the PCA plot.

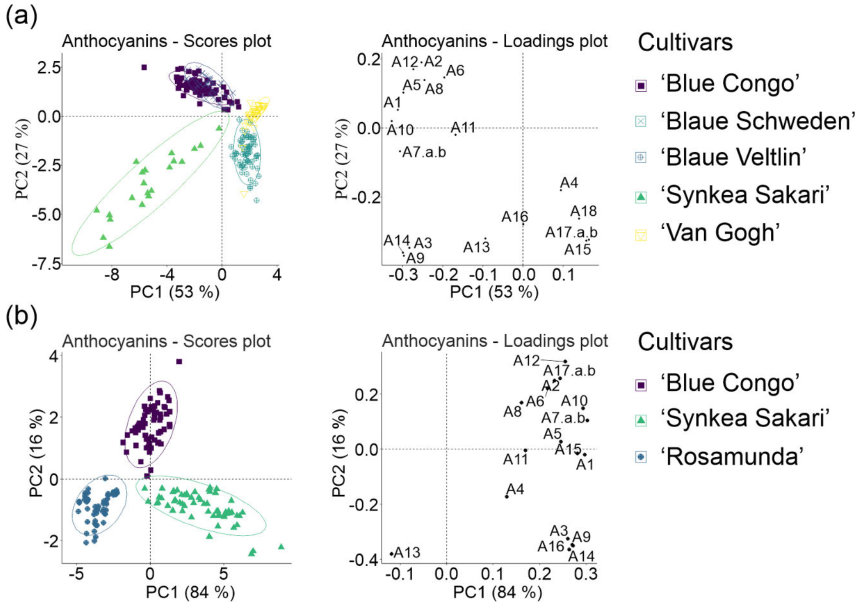


Fig. 17 Scores and loadings plot of the ANOVA-simultaneous component analysis (ASCA) model based on the effect of “Cultivar” on the anthocyanin profile in: (a) samples cultivated in a greenhouse at controlled temperatures (**Study II**); (b) samples cultivated in the field trials (**Study III**).

ASCA models could clearly separate the samples from the pigmented cultivars (‘Blue Congo’, ‘Synkeä Sakari’, and ‘Rosamunda’) that grew in the field trials (Study III). PC1 of the ASCA model explained 84 % and PC2 16 % of the variance **(Fig. 17b)**. ‘Synkeä Sakari’ had the highest content of most of the anthocyanins and thus is located on the right side of PC1. ‘Rosamunda’ (the red-skinned cultivar with yellow flesh) had the lowest content of anthocyanins, its main anthocyanin being pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside (A13). Therefore, it is clearly differentiated from the purple-fleshed cultivars and is located on the left side of PC1. Moreover, ‘Synkeä Sakari’ had the highest content of anthocyanins A3, A9, A16 and A14, thus the samples of this cultivar are located on the lower right side of the plot.

Other studies that compared the anthocyanin profiles of pigmented potato cultivars, have shown that purple or blue-fleshed potatoes have petunidin-3-*p*-coumaroylrutinoside-5-glucoside or malvidin-3-*p*-coumaroylrutinoside-5-glucoside as the main anthocyanins, whereas red-fleshed potatoes have pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside as the main anthocyanin (Giusti & Wrolstad, 2001; Hillebrand et al., 2008; Lachman et al., 2009; Lewis et al., 1998).Thus, the genetic background of the potato cultivar determines the anthocyanin profile.

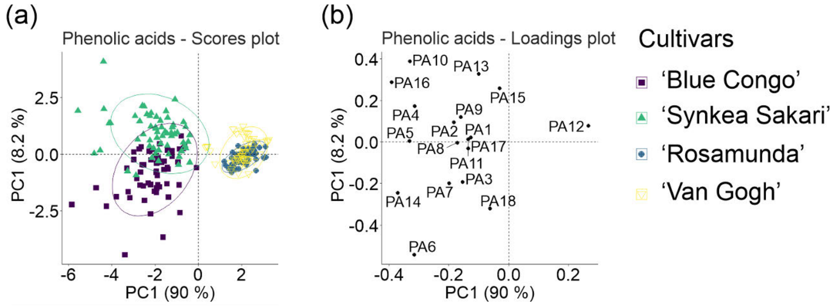


Fig. 18 Scores and loadings plot of the ANOVA-simultaneous component analysis (ASCA) model based on the effect of “Cultivar” on the phenolic acid profile in: (a) samples cultivated in a greenhouse at controlled temperatures (**Study II**); (b) samples cultivated in the field trials (**Study III**).

ASCA model showed that the phenolic acid profile varied according to the flesh color of the cultivar (**Fig. 18**). The yellow-fleshed cultivars had one phenolic acid that was at higher proportion than in the purple-fleshed cultivars (PA12, λmax= 321 nm, unidentified phenolic acid). The phenolic acid profile was relatively similar in the purple-fleshed cultivars ‘Synkeä Sakari’ and ‘Blue Congo’.

At full maturity, cultivar determined the total amount of anthocyanins, phenolic acids and ascorbic acid that the plant can produce. ‘Synkeä Sakari’ produced the highest anthocyanin content followed by ‘Blaue Veltlin’ (**Fig. 19a**). ‘Blue Congo’ and ‘Blaue Schweden’ showed the lowest anthocyanin content and there was not significant difference between these two cultivars when grown at controlled temperatures (**Study II**). ‘Synkeä Sakari’ and ‘Blaue Veltlin’ had similar amounts of total phenolic acids and these values were higher than in ‘Blue Congo’ and ‘Blaue Schweden’ which, again, did not show significant differences in the content of phenolic acids (**Fig. 19b**). The content of ascorbic acid varied slightly between the cultivars, but the difference was not significant (**Fig. 19c**).

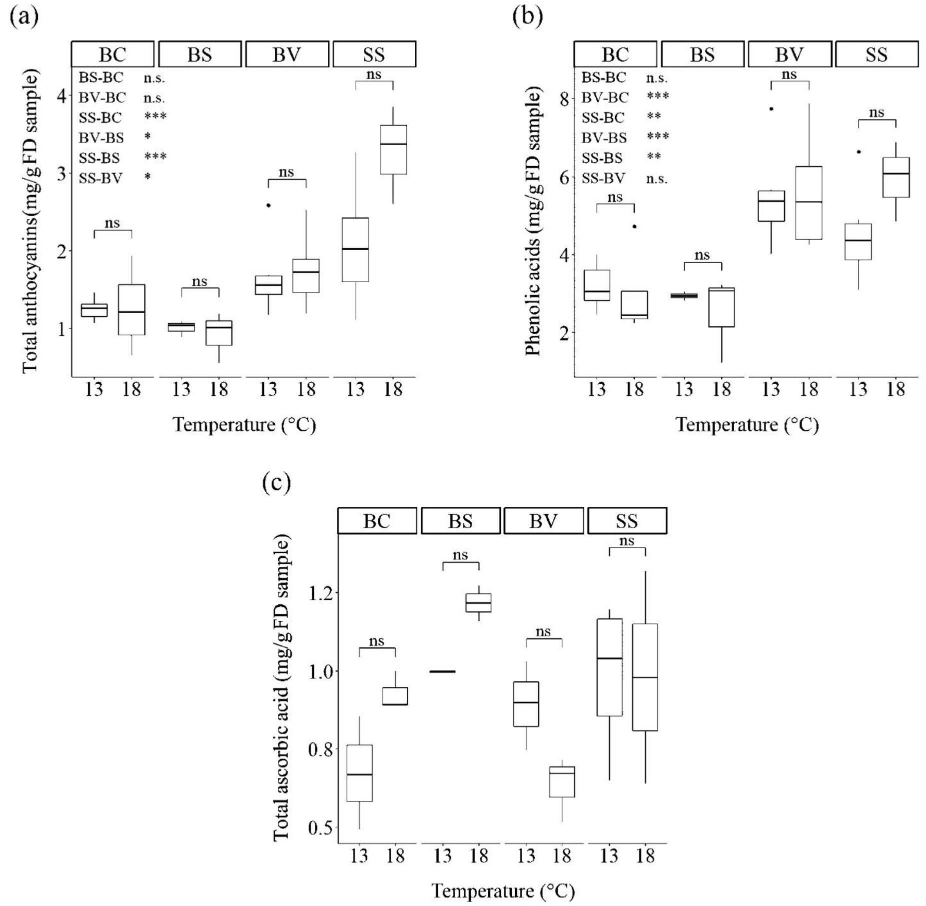


Fig. 19 Content of total anthocyanins, phenolic acids and ascorbic acid in fully mature tubers harvested at 120 DAP (except for BS which was harvested at 110 DAP). BC: ‘Blue Congo’, BS: ‘Blaue Schweden’, BV: ‘Blaue Veltlin’, SS: ‘Synkeä Sakari’. *p* values from Tukey's test are shown of the left upper part of each plot ($p < 0.001$: ***; $p < 0.01$: **; $p < 0.05$: *; ns: not significant). t-tests with unequal variance or equal variance (depending on the case) were used to compare the significant changes in the anthocyanin, phenolic acids and ascorbic acid content when tubers were cultivated at 13 and 18 °C. *p* values from the t-tests are shown above each pair compared (**Study II**).

Additionally, it was found that the cultivars with the highest content of anthocyanins also had the highest content of phenolic acids (**Fig. 20**). This positive correlation between the content of anthocyanins and phenolic acids has also been reported in other studies (Lewis et al., 1998; Payyavula et al., 2012).

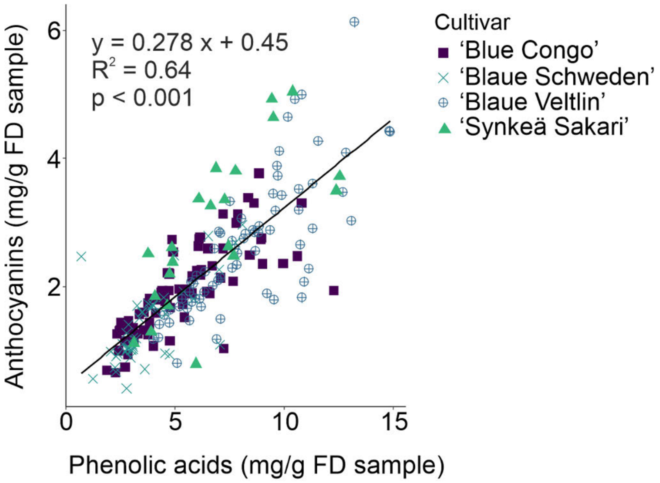


Fig. 20 Pearson correlation between the total content of anthocyanins and phenolic acids in purple-fleshed potato cultivars grown at 13 and 18 °C and collected from 60 to 120 DAP (**Study II**). Data represents the values of individual plant replicates. Reprinted from the original publication **II** with permission from Elsevier.

5.3 Effect of tuber developmental stage on tuber yields and on the synthesis of anthocyanins, phenolic acids and ascorbic acid in potato tubers (Study II and Study III)

5.3.1 Effect of tuber developmental stage on crop yield

Naturally, potato tuber yield increased throughout tuber development in all cultivars studied, however, the number of tubers produced per plant remained relatively stable during tuber development (**Fig. 21**). It has been shown in previous studies that the number of incipient tubers increased in the early stages of tuber development (from 0 to 45 DAP) and once they reached 60 g (around 45 DAP) the number of tubers remained stable throughout plant development (Struik, 2007a). The authors also found that when plants produced many incipient tubers, not all of them reached marketable size. Instead, some of them were resorbed during the growing season, resulting in a decrease in the number of tubers. We observed the same in **Study III**, the number of tubers remained relatively stable during tuber bulking, but in some cases, it decreased towards the end of tuber maturity.

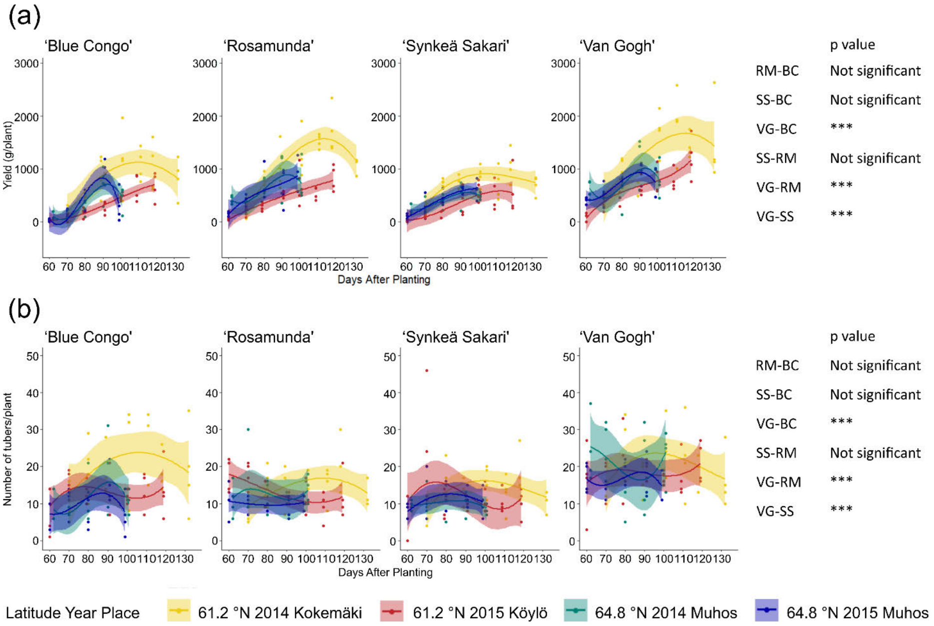


Fig. 21 Spline regression of the changes in: (a) Yield, and (b) Number of tubers produced at different stages of tuber development in potato cultivars grown at two different latitudes, years and places. The colored ribbons show the standard deviation (n = 4). p values from the Tukey's test are shown on the right side ($p < 0.001$: ***). BC: 'Blue Congo', RM: 'Rosamunda', SS: 'Synkeä Sakari', VG: 'Van Gogh' (**Study III**).

5.3.2 Effect of tuber developmental stage on the expression levels of the genes involved in the phenylpropanoid pathway

When potato tubers were cultivated in a greenhouse under controlled temperatures (**Study II**), the expression levels of most of the genes studied did not show a clear tendency to either increase or decrease. However, the expression levels of *PAL* were very low at 60 DAP and slightly increased during tuber bulking, while the expression levels of *F3´H* were already low at 60 DAP and decreased during tuber bulking. When potato tubers were cultivated on the fields in different years and latitudes (**Study III)**, again the expression levels of most of the genes did not show a clear increasing or decreasing trend. However, the expression levels of *F3´H*, which were already low, showed a clear decreasing trend ($p < 0.001$) in all the purple-fleshed cultivars ('Blue Congo', 'Synkeä Sakari') and a mild decreasing trend ($p < 0.01$) in the yellow-fleshed cultivar ('Van Gogh') (**Fig. 22**).

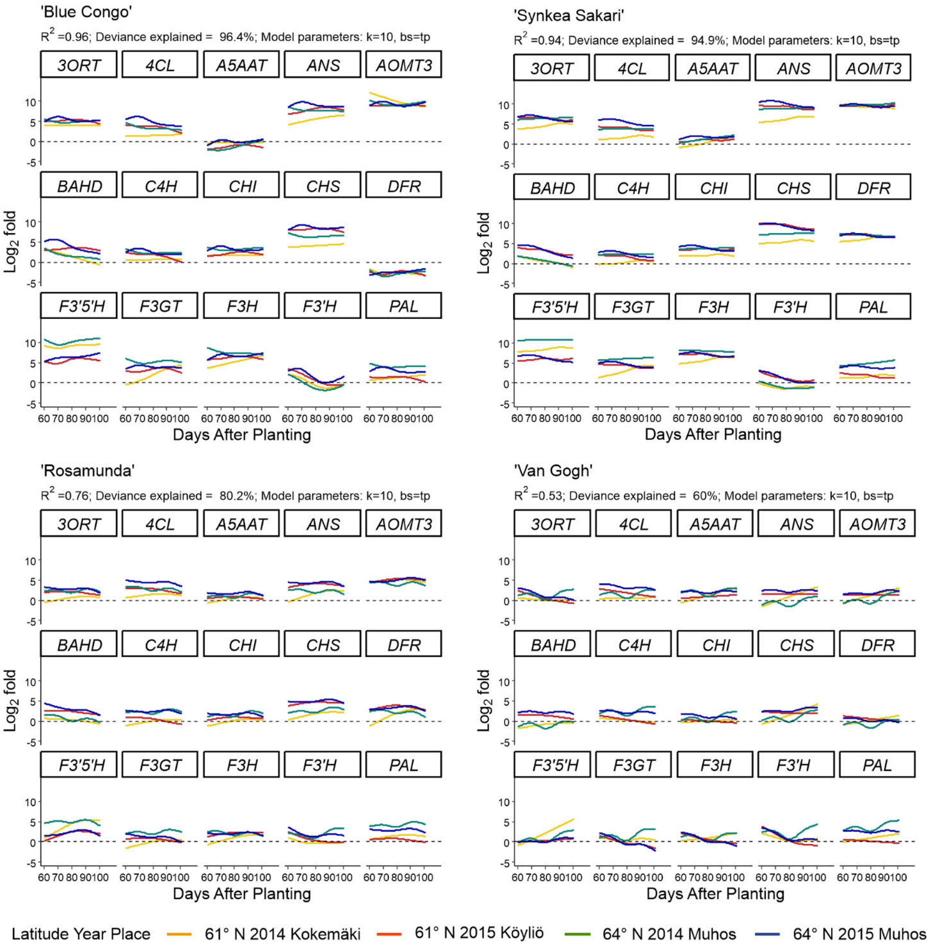


Fig. 22 Spline regression using GAMs on the gene expression levels along tuber development (**Study III**).

Information about the changes in expression levels of the genes involved in the phenylpropanoid pathway during potato tuber development is scarce and contradicts with our results. Two studies performed on potato tubers collected from 60 to 170 days after planting (DAP), showed that the expression levels of *C4H*, *PAL*, *DFR*, *F3GT* (also known as *UFGT*), *F3H* and *ANS* decreased during tuber development (Navarre et al., 2013; Payyavula et al., 2013). The authors also found that the expression levels of those genes and the content of the main phenolic compounds (including anthocyanins) decreased during maturation. It is worth noticing that the data they presented were based on a different method to calculate the gene expression levels (Ct method), while in our studies the resulting folds were log$_2$-transformed.

In our study, the genes with continuously high expression levels during tuber development in all the field trials in ‘Synkeä Sakari’ and ‘Blue Congo’ were *ANS*, *AOMT3*, *F3′5′H* and *F3H*. In ‘Rosamunda’, *AOMT3* was expressed at high levels while in ‘Van Gogh’ most genes were down-regulated and gene expression increased only slightly depending on the trial field (**Fig. 23**).

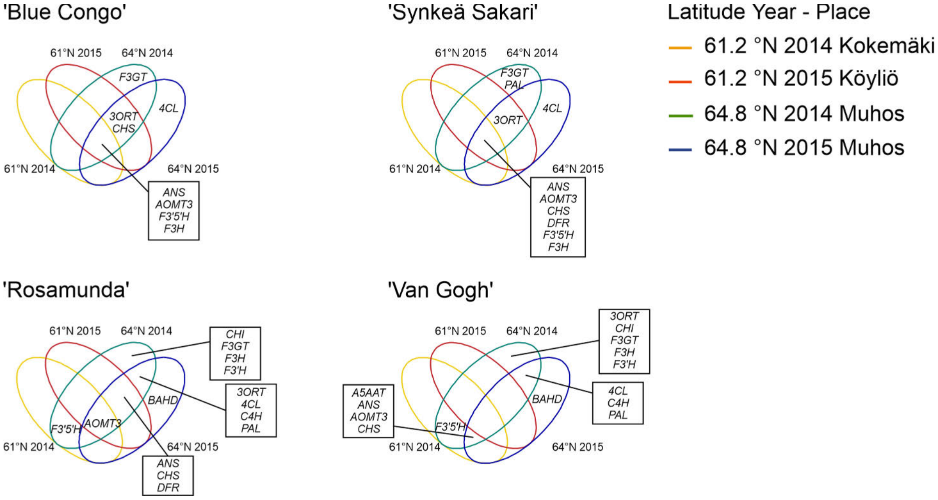


Fig. 23 Venn diagrams showing the genes with higher gene expression levels along tuber development (**Study III**).

5.3.3 Effect of tuber developmental stage on the content of anthocyanins, phenolic acids and ascorbic acid

In **Study II**, we found that the main anthocyanins in the purple-fleshed cultivars decreased during tuber development, but minor anthocyanins, such as pelargonidin, peonidin and cyanidin derivatives, showed relatively stable levels during tuber development (**Fig. 24**). We found that a few samples of the yellow-fleshed cultivar ‘Van Gogh’ had small areas with a slight pink coloration. Subsequent chemical analysis showed that these samples contained tiny amounts of anthocyanins, which neither increased nor decreased during tuber development.

In **Study II**, unbalanced ANOVA and Dunnet tukey test showed that young tubers harvested at 70 DAP had significantly higher content of anthocyanins than matured tubers harvested from 100 to 120 DAP.

In **Study III**, spline regression showed that, again, minor anthocyanins did not show a clear trend to increase or decrease. Only the main anthocyanins decreased with maturation. General additive models (GAM) were used to evaluate the changes in the content of total anthocyanins, total phenolic acids and total ascorbic acid. The model showed that in general the concentrations of total anthocyanin, phenolic acids and ascorbic acid decreased during tuber bulking from 60 to 120 DAP (**Fig. 25**, **Fig. 26**). The “DAP:Latitude” and “DAP:Year” interaction also affected the production of these compounds. Thus, potato samples grown at 64.8° N in 2015 (Muhos) showed a low content of total anthocyanins, phenolic acids and ascorbic acid at 60 DAP and clearly increased at 80 DAP, but subsequently decreased or remained stable during tuber development. This delay in the production of phenolic compounds and ascorbic acid was especially prominent in the cultivar 'Blue Congo'.

Other studies have also observed a decrease in anthocyanin and phenolic acid content during tuber maturation (Navarre et al., 2013; Payyavula et al., 2013; Reyes et al., 2004). There is only one study concerning the effect of tuber developmental stage on the content of ascorbic acid. This study reported that the content of ascorbic acid increased from non-tuberized stolons up to fully developed tubers (140 DAP) and decreased slightly at 160 DAP (Blauer et al., 2013).

Additionally, the total anthocyanin, total phenolic acid and total ascorbic acid yield that a plant could produce at different stages of tuber development was evaluated for the samples collected at 64 N° in 2015 (Muhos). The data showed that the content of anthocyanins, phenolic acids and ascorbic acid increased mildly at the final stage of tuber development. However, after performing one-way ANOVA and Tukey post hoc test, the results showed that the increment was not significant in most of the cases, especially in 'Rosamunda', 'Synkeä Sakari' and 'Van Gogh' (**Fig. 27**).

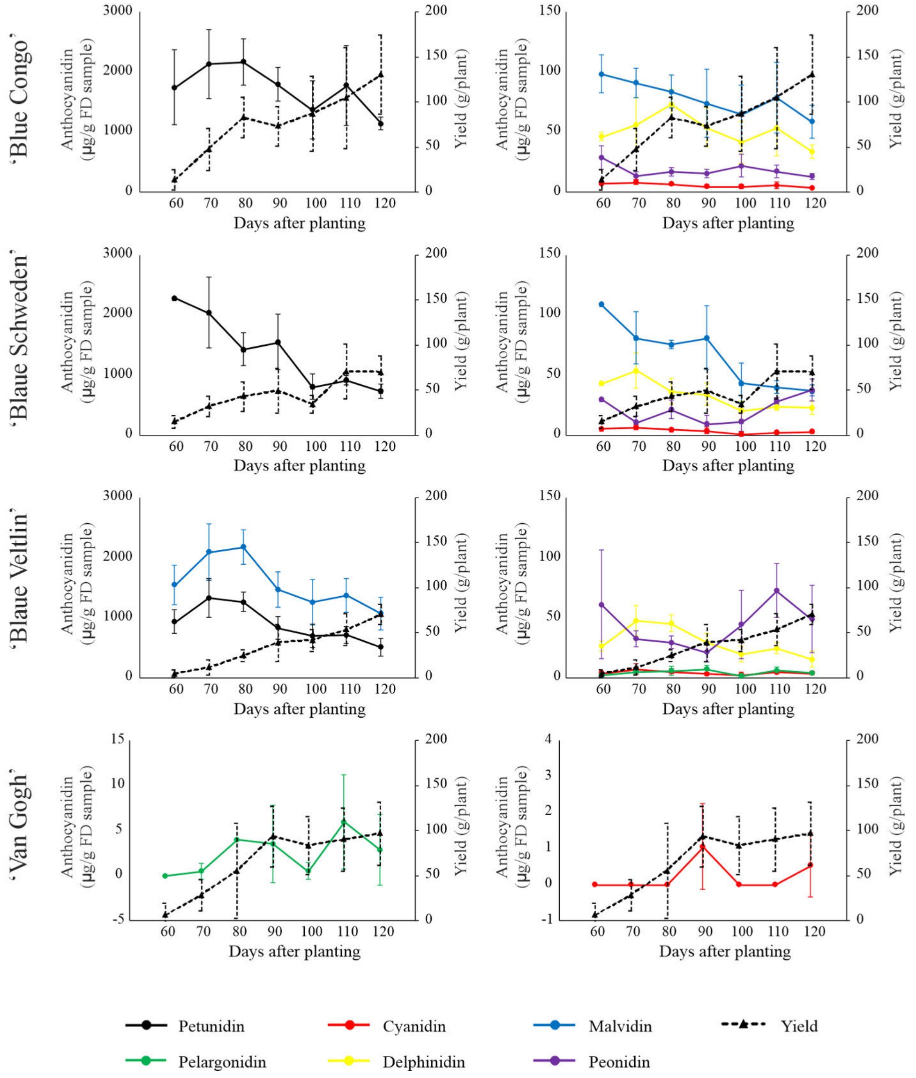


Fig. 24 Changes in the content of glycosides of individual anthocyanidins during tuber development in four potato cultivars grown at 13 °C. Error bars show the standard deviation between individual plant replicates. The cultivar 'Synkeä Sakari' was excluded from the figure because the tuber production was insufficient from 60 to 80 DAP for obtaining adequate biological replicates. Reprinted from the original publication **II** with permission from Elsevier.

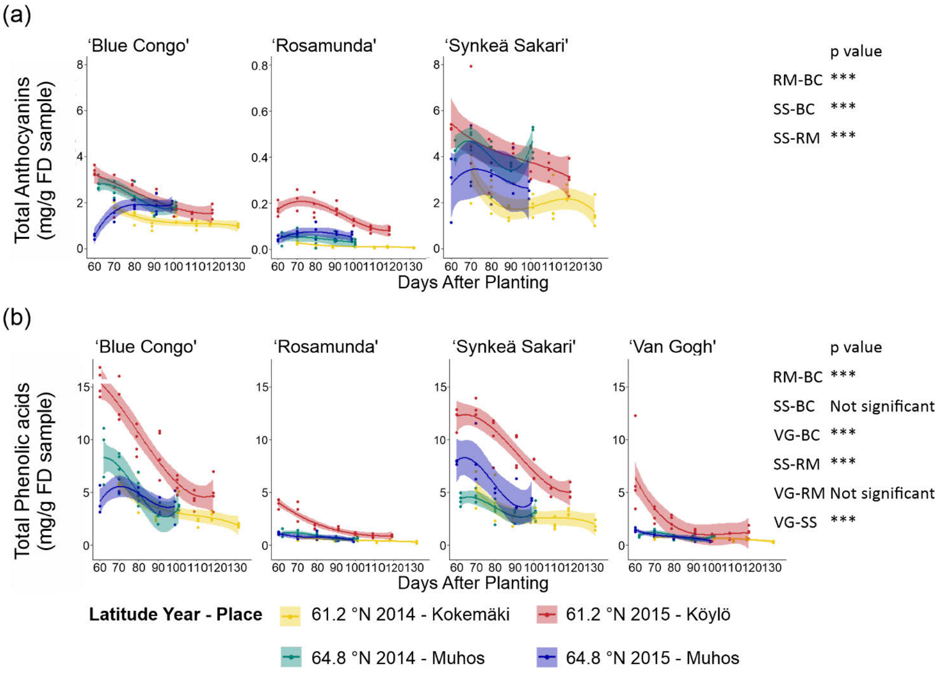


Fig. 25 Spline regression of the changes in: (a) Total anthocyanins, and (b) Total phenolic acids at different stages of tuber development in potato cultivars grown at two different latitudes, years and places. The colored ribbons show the standard deviation (n=4). p values from the Tukey's test are shown on the right side ($p < 0.001$: ***; $p < 0.01$: **; $p < 0.05$: *). BC: 'Blue Congo', RM: 'Rosamunda', SS: 'Synkeä Sakari', VG: 'Van Gogh' (**Study III**).

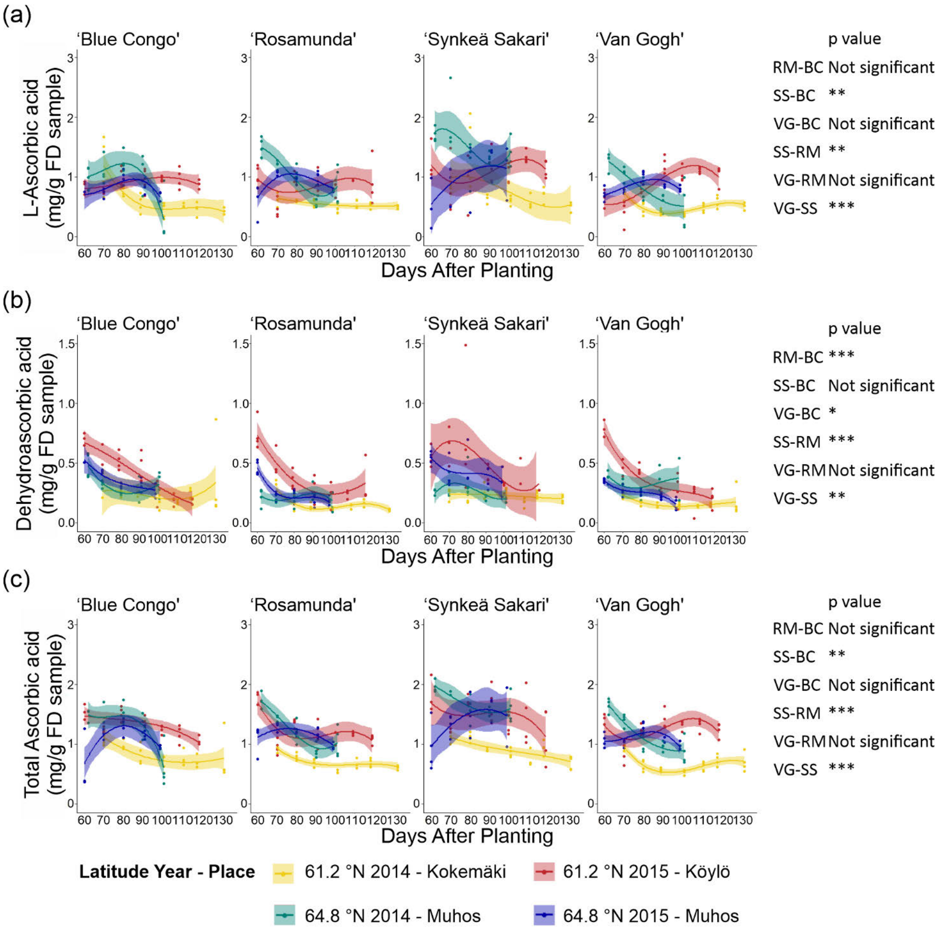


Fig. 26 Spline regression of the changes in: (a) L-ascorbic acid, (b) dehydroascorbic acid and (c) total ascorbic acid at different stages of tuber development in potato cultivars grown at two different latitudes, years and places. The colored ribbons show the standard deviation (n=4). p values from the Tukey's test are shown on the right side ($p < 0.001$: ***; $p < 0.01$: **; $p < 0.05$: *). BC: 'Blue Congo', RM: 'Rosamunda', SS: 'Synkeä Sakari', VG: 'Van Gogh' (**Study III**).

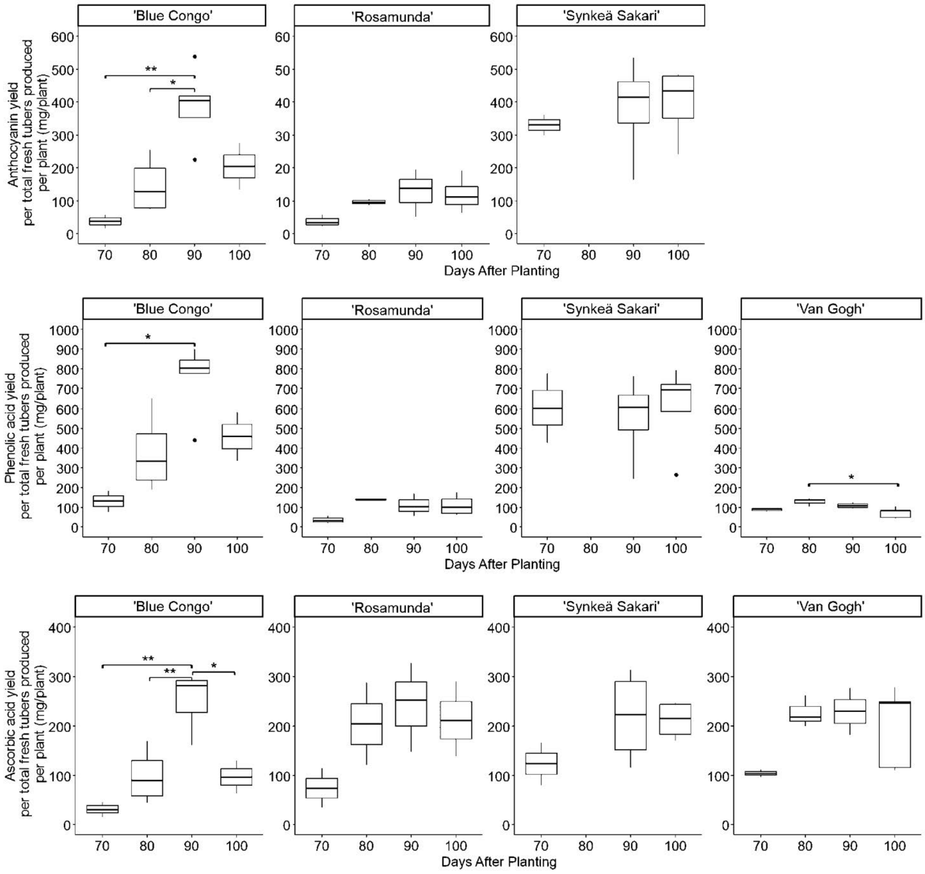


Fig. 27 Box plots of the anthocyanin, phenolic acid, and ascorbic acid yield produced per plant at different stages of tuber development in potato cultivars grown at 64° N in 2015 (Muhos) (n=2 to 4). Significant differences found after performing Tukey's test are shown on the upper part of the box plots ($p < 0.001$: ***; $p < 0.01$: **; $p < 0.05$: *) (**Study III**).

5.4 Effect of weather conditions on tuber yield and on the synthesis of anthocyanins, phenolic acids and ascorbic acid in potato tubers (Study II and Study III)

5.4.1 Effect of controlled temperatures on the content of anthocyanins, phenolic acids and ascorbic acid (Study II)

The optimal temperature for tuber induction is 15 °C and for tuber bulking is between 14 and 22 °C (Struik, 2007b). Thus, 18 °C was selected as an optimal temperature for tuber growth and 13 °C as a mild cool temperature that could enhance the synthesis of phenolic compounds. However, when potato tubers were cultivated under these controlled temperature conditions (13 and 18 °C)

(**Study II**), these temperatures did not have a significant effect on the content of total anthocyanins, phenolic acids or ascorbic acid in fully matured tubers (**Fig. 19**).

5.4.2 Weather conditions that characterized the field trials at different latitudes and years (Study III)

ASCA models of the weather data showed that meteorological conditions in the field trials were different between latitudes and years (**Fig. 28**). PC1 explained 67 % of the variance which was mainly caused by the precipitation events. The field trials of 2015 were exposed to higher precipitation events than the field trials of 2014. On the other hand, the field trials performed in 2014 were exposed to higher temperatures and global radiation than in 2015. PC2 explained 29 % of the variance which was mainly due to wind and gust and speed. The trials at 61.2 °N (Köyliö and Kokemäki) were characterized by higher wind gusts and speed than the trials located at 64.8 °N (Muhos).

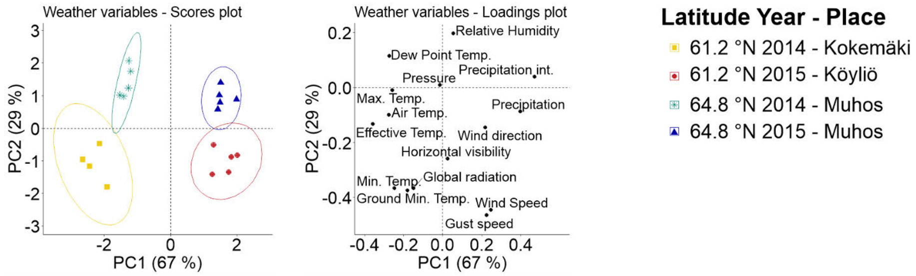


Fig. 28 Scores and loadings plot of the ANOVA-simultaneous component analysis (ASCA) model based on the effect of "Latitude – Year – Place" on the cumulative values (from 60 to 100 DAP) of the weather conditions present in the different cultivation trials (**Study III**).

GAMs helped to understand how the main weather variables evolved during the growing seasons. GAMs also revealed that the weather conditions in the field trials were different during the growing seasons. The trial at 61.2° N in 2014 (Kokemäki) was exposed to the highest temperatures after 60 DAP (**Fig. 29a**). This trial had 6 days with maximum temperatures above 30 °C during the growing season. This trial also had the lowest precipitation events from 0 to 80 DAP increasing only after 80 DAP (**Fig. 29b**). This trial was also exposed to the highest global radiation after 60 DAP (**Fig. 29c**). The trial 61.2° N in 2015 (Köyliö) was exposed to temperatures between 13 and 18 °C during tuber bulking (60 to 100 DAP) (**Fig. 29a**). The trial 64.8° N in 2015 (Muhos) was also exposed to temperatures between 13 and 18 °C during most of the tuber bulking stage (**Fig. 29a**), but was the one that received the lowest global radiation (**Fig. 29c**). The trial 64.8° N in 2014 (Muhos) was exposed to temperatures above

18 °C in the early stages of tuber formation (from 30 to 60 DAP) and quickly dropped below 13 °C after 70 DAP (**Fig. 29a**).

The main weather variables that affect the crop evapotranspiration are radiation, air temperature, humidity and wind speed (Allen et al., 1998). The crop evapotranspiration determines the water requirement and therefore impacts the growth and yield of the crop. It was found that due to climate change, temperatures in China have increased in the past 60 years and that the reference crop evapotranspiration (ET_o) has also increased significantly after 1985 (Fan et al., 2016). Thus, the crop water demand will increase due to climate change.

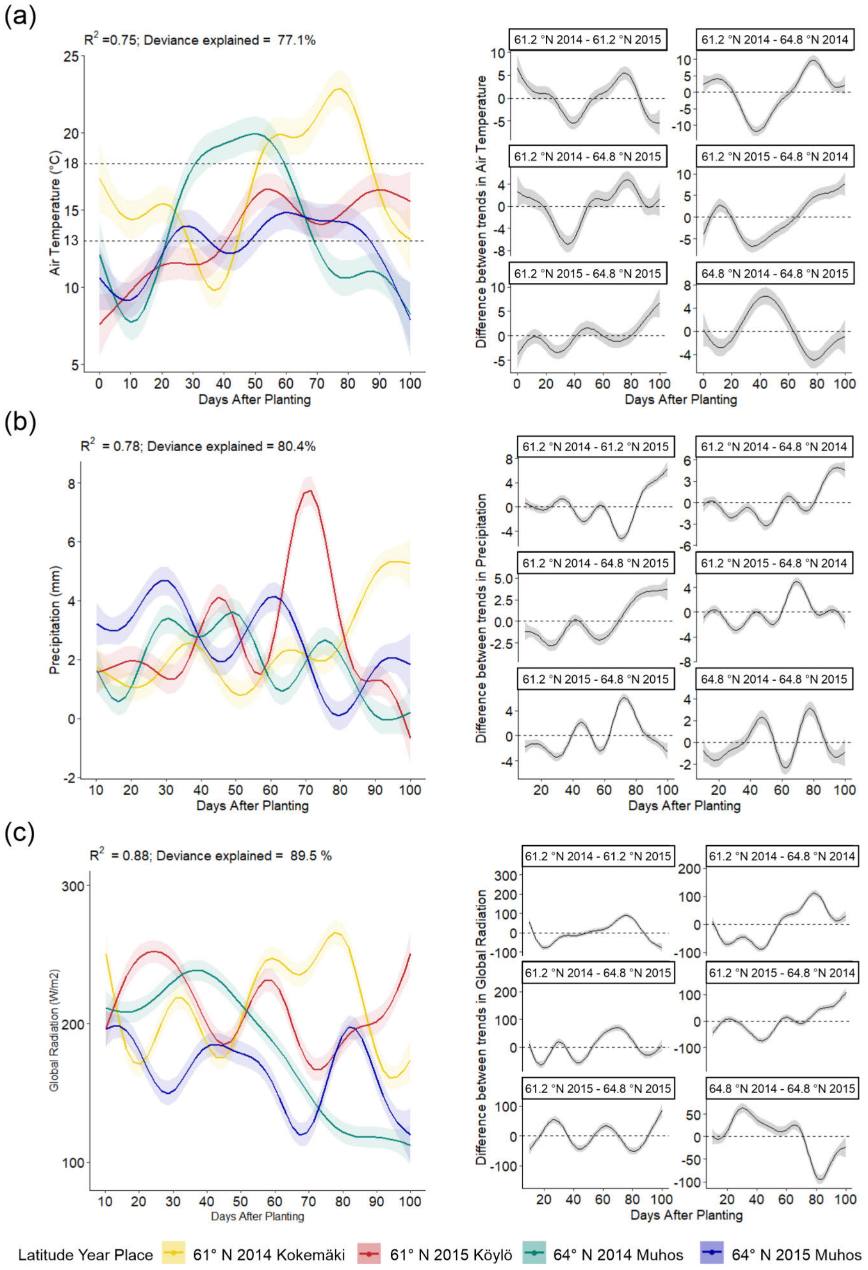


Fig. 29 Fitted smooth functions using GAMs (left plots) and differences between the fitted smooth functions (right plots). (a) Splines for daily air temperatures, (b) Splines after performing simple moving average (period of 10 days) of daily precipitation, (c) Splines after performing simple moving average (period of 10 days) of daily global radiation. The colored and grey ribbons show the confidence intervals. If the grey ribbons excludes zero, there are significant differences between the pair of estimated smooths (**Study III**).

5.4.3 Effect of latitude and year on the content of anthocyanins, phenolic acids and ascorbic acid in potato tubers grown in the fields (Study III)

Unbalanced three-way ANOVA showed that the effect of "Latitude", "Year" and the interaction "Latitude:Year" produced significant changes in the gene expression levels of the genes involved in the phenylpropanoid pathway, as well as the content of anthocyanins, phenolic acids and ascorbic acid.

5.4.3.1 Effect of latitude and year on the gene expression profiles

ASCA models showed that gene expression profiles in the purple-fleshed cultivars ('Blue Congo' and 'Synkeä Sakari') grown at 61.2° N in 2014 (Kokemäki) were clearly discriminated from the gene expression profiles of the tubers produced in the other field trials. PC1 of the ASCA models in the purple-fleshed cultivars explained 62 and 64 % of the variance in 'Blue Congo' and 'Synkeä Sakari', respectively (**Fig. 30a; b**). The main gene causing the variance in PC1 was *ANS*. The tubers that grew in Kokemäki showed the lowest *ANS* expression levels (**Fig. 22**). PC2 explained 33 and 31 % of the variance in 'Blue Congo' and 'Synkeä Sakari', respectively (**Fig. 30a; b**). The main genes causing the variance in PC2 were *F3'5'H* and *BAHD* which varied according to the year of cultivation. The gene expression levels of *F3'5'H* were higher in 2014 than in 2015, while the opposite occurred with *BAHD*. *BAHD* expression levels were higher in tubers produced in 2015 than in 2014 (**Fig. 22**).

ASCA model in the red-skinned cultivar ('Rosamunda') discriminated the samples mainly according to the year of cultivation while only mild differences were found between latitudes. PC1 in the red-skinned cultivar explained 60 % of the variance, which was mainly caused by *4CL*, *CHS* and *BAHD*. The samples with the highest gene expression levels were located on the right side of the plot **Fig. 30**). PC2 explained 36 % of the variance mainly caused by *PAL* followed by *F3'5'H*. In 2014, *F3'5'H* expression levels in 'Rosamunda' tubers were higher than tubers grown in 2015 (**Fig. 22**). In the case of PAL, higher expression levels were seen in the tubers grown at 64.8 °N than at 61.2 °N.

On the other hand, the gene expression levels in the yellow-fleshed cultivar ('Van Gogh') showed only mild differences in the gene expression levels of the samples harvested in the different cultivation trials. The ASCA model could not discriminate well the samples produced in the different trials. PC1 of the ASCA model explained 50 % of the variance which was mainly caused by PAL and C4H (**Fig. 30d**). In general, in this cultivar, tubers produced in the higher latitude zone (64.8° N) had higher expression levels of 4CL, C4H and PAL than tubers produced in the lower latitude zone (61.2° N) (**Fig. 23**). Moreover, PC2 explained 41 % of the variance and showed that the tubers grown at 64.8° N in

2015 (Muhos) had higher BAHD expression levels than the tubers grown in the other trials (**Fig. 23**, **Fig. 30d**).

In general, the pigmented cultivars that grew in Kokemäki were exposed to the highest temperatures, and the lowest precipitation events during tuber development and produced tubers with reduced expression levels of the genes studied. Thus, heat stress and drought down-regulated the expression levels of the genes involved in the phenylpropanoid pathway. A study that exposed mature, red-skinned potato tubers to heat stress for one week prior to harvest reported down-regulation of most genes involved in phenylpropanoid metabolism and a reduction in reddish skin color of the tubers (Fogelman et al., 2019). Likewise, a study with a purple flesh cultivar cultivated at two different controlled temperatures reported down-regulation of the genes involved in the phenylpropanoid pathway in the tubers exposed to the highest temperatures (Y. Liu et al., 2019). A study in different potato cultivars reported that genes involved in the central phenylpropanoid pathway (*PAL* and *C4H*), and flavonoid biosynthetic genes (except *F3'H*) were down-regulated when exposed to drought conditions (André et al., 2009).

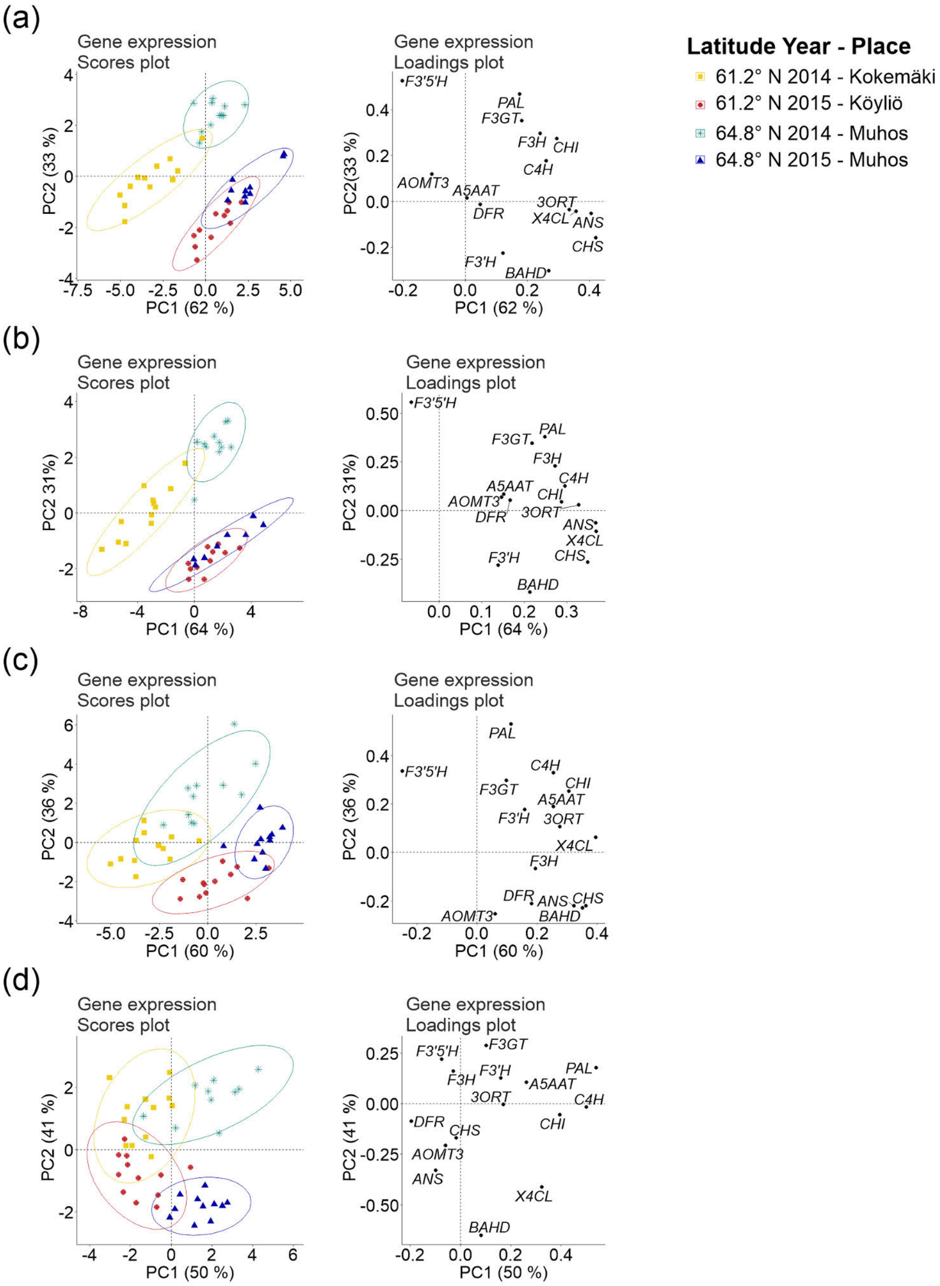


Fig. 30 Scores and loadings plot of the ANOVA-simultaneous component analysis (ASCA) model based on the effect of "Latitude – Year – Place" on the gene expression levels of the cultivars: a) 'Blue Congo', b) 'Synkeä Sakari', c) 'Rosamunda', and d) 'Van Gogh' harvested at different stages of tuber development (from 70 to 100 DAP) (**Study III**).

5.4.3.2 Effect of latitude and year on the content of anthocyanins

Latitude and year affected the anthocyanin profile of the pigmented cultivars. Loading plots of the ASCA model in the pigmented cultivars showed that most of the anthocyanins are located on the right side of the plots. Thus, the samples with the highest anthocyanin content tended to be on the right side of the plots (**Fig. 31**).

ASCA model in ‘Blue Congo’ (**Fig. 31a**) was able to discriminate mainly the samples produced at 61.2° N in 2014 (Kokemäki) from the samples produced in the other trials. The samples from Kokemäki had the lowest anthocyanin contents and were therefore located on the left side of the plot.

In ‘Synkeä Sakari’ (**Fig. 31b**) PC1 revealed that 67 % of the variance was mainly caused by anthocyanins A2 and A5 (petunidin derivatives), which were the highest in the cultivation trial 64.8° N in 2014 (Muhos), while PC2 revealed that 27 % of the variance was mainly caused by anthocyanin A11 (a peonidin derivative) which was the highest in the cultivation trial 61.2° N in 2015 (Köyliö).

In ‘Rosamunda’ (**Fig. 31c**) PC1 showed most of the variance (98 %). This model could clearly discriminate the samples produced at 61.2° N in 2015 (Köyliö) from the other field trials. These samples had the highest anthocyanin contents.

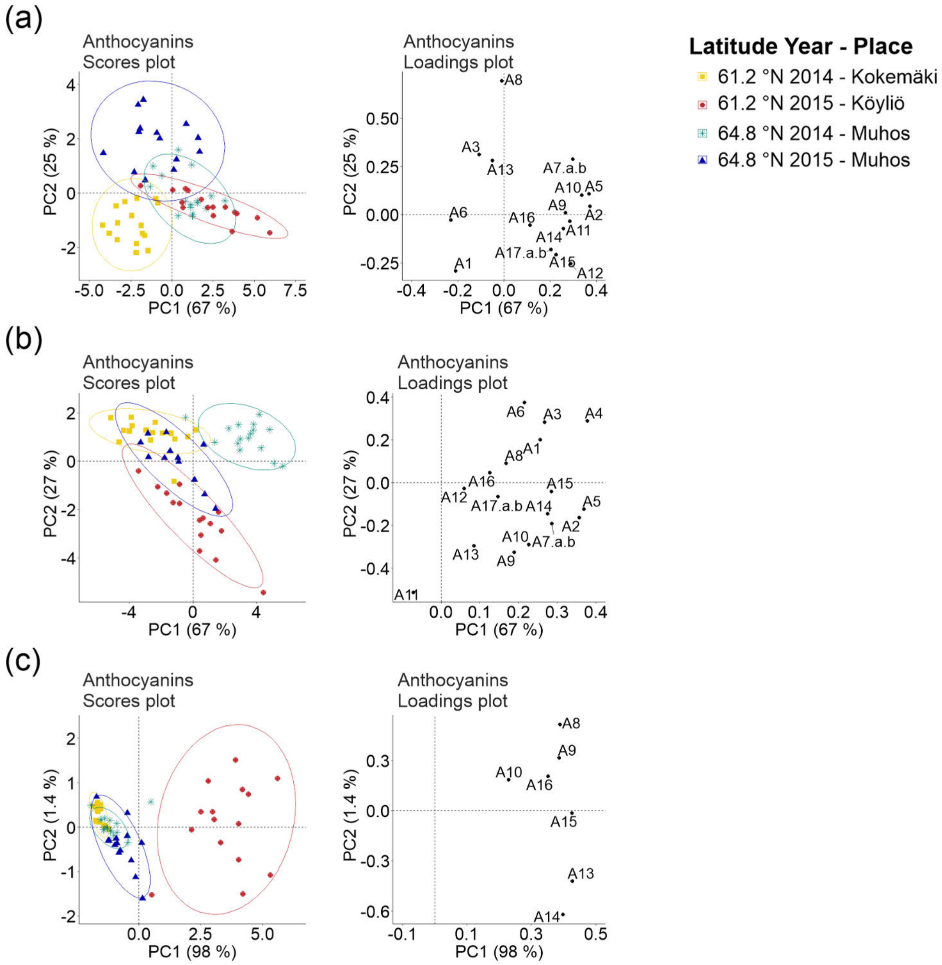


Fig. 31 Scores and loadings plot of the ANOVA-simultaneous component analysis (ASCA) model based on the effect of "Latitude – Year – Place" on the anthocyanin profile of the cultivars: a) 'Blue Congo', b) 'Synkeä Sakari', and c) 'Rosamunda' harvested at different stages of tuber development (from 70 to 100 DAP) (**Study III**).

Studies performed under controlled conditions revealed reduced anthocyanin content in potato tubers exposed to heat stress (Fogelman et al., 2019; Y. Liu et al., 2019). Moreover, field studies at different latitudes in the USA showed higher content of anthocyanins in potato tubers grown between 58 and 67° N (Alaska) than in those grown between 29 and 33° N (Florida and Texas, respectively) (Payyavula et al., 2012). Another field study performed in Colorado (37° N) and Texas (25° N) reported that potato tubers grown in Colorado had higher content of anthocyanins than the tubers grown in Texas. This was because the potatoes in Colorado were exposed to higher global radiation, longer days and lower temperatures (especially during tuber bulking) than the potatoes grown in Texas (Reyes et al., 2004). The content of anthocyanins in potato cultivars grown under dry conditions was reduced in comparison to the cultivars grown under irrigated conditions (Andre et al., 2009).

In our study, the tubers produced in Köyliö were exposed to temperatures in the range of 13 to 18 °C and produced tubers with the highest anthocyanin content. The tubers in Kokemäki were exposed to the highest growing temperatures and the lowest precipitation events and produced tubers with the lowest anthocyanin content.

It is also important to mention that the tubers from Kokemäki had skin lesions caused by scabs (produced by *Streptomyces scabies*, *S. turgidiscabies* and other *Streptomyces* species). This disease produces the toxin thaxtomin A which causes inhibition of cellulose synthesis and affects the expression of genes involved in primary and secondary cell wall synthesis (Johnson et al., 2009). Down-regulation of the genes involved in the primary cellulose biosynthesis and the 4-coumarate:CoA ligase gene (specifically 4CL3) were down-regulated in *Arabidopsis thaliana* seedlings treated with 200 nM thaxtomin A (Bischoff et al., 2009). This disease may be prevented by irrigation during the early stages of tuber development (Johansen et al., 2015; Lapwood et al., 1971). Therefore, the high temperatures and dry conditions in the field trials in Kokemäki could not only cause lower anthocyanin content but also higher susceptibility of the potato cultivars to the infestation by Streptomyces species on the potato skins which at the same time would affect the expression of the genes involved in the phenylpropanoid pathway.

5.4.3.3 Effect of latitude and year on the content of phenolic acids

ASCA models could not find marked differences in the phenolic acid profile in the tubers produced at 64.8 °N in 2014 and 2015. However, the models showed marked differences between tubers harvested at 61.2° N in 2014 and 2015. PC1 explained between 59 and 74% of the variance between cultivars while PC2 explained between 21 and 27 % of the variance which was mainly caused by PA5 (chlorogenic acid) (**Fig. 32**). Tubers from all the pigmented cultivars (‘Blue Congo’, ‘Synkeä Sakari’ and ‘Rosamunda’) collected at 61.2° N (Köyliö) in 2015 had the highest content of PA5 and tended to be in the lower right side of the plot.

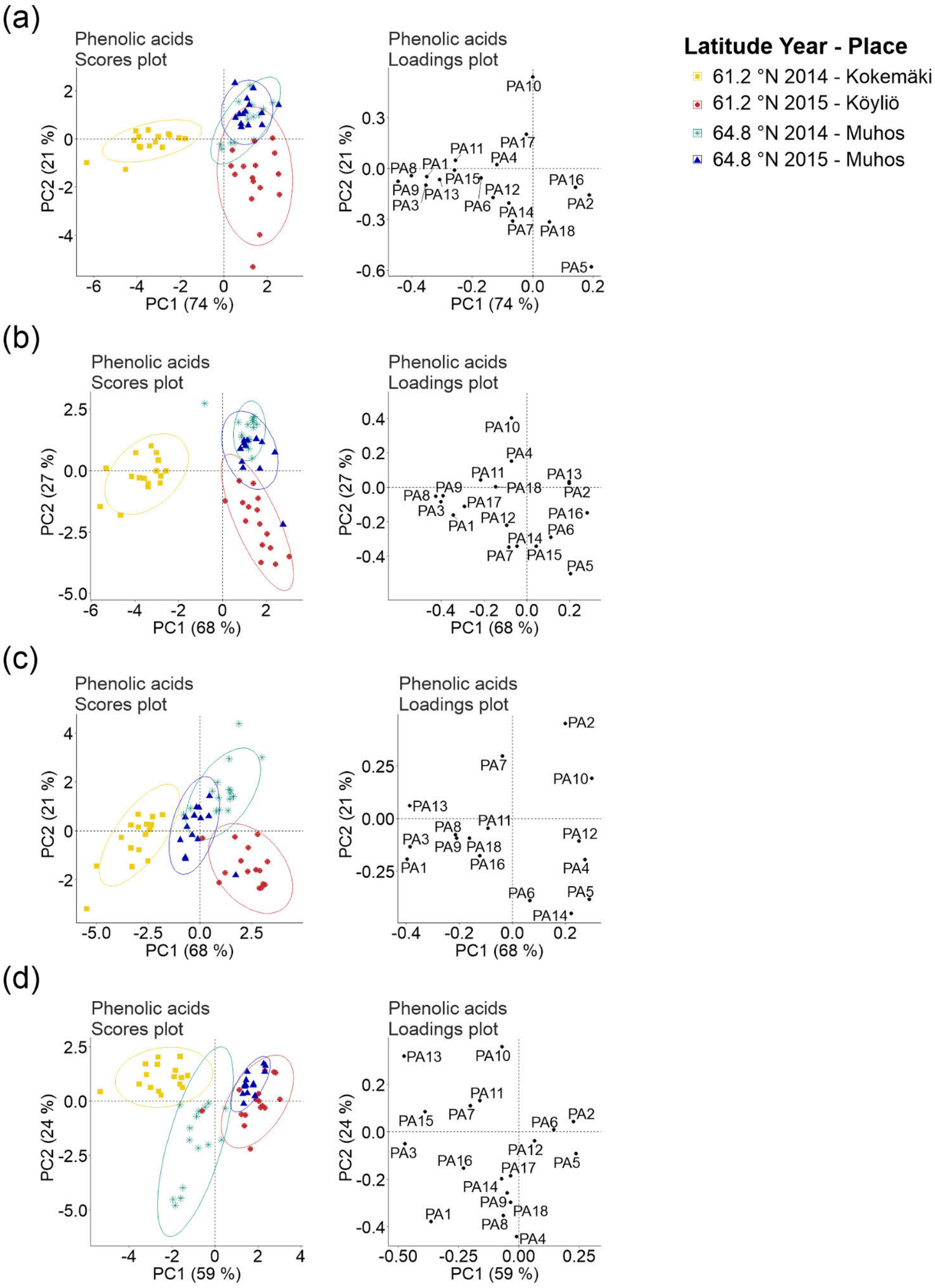


Fig. 32 Scores and loadings plot of the ANOVA-simultaneous component analysis (ASCA) model based on the effect of "Latitude – Year – Place" on the phenolic acid profile of the cultivars: a) 'Blue Congo', b) 'Synkeä Sakari', c) 'Rosamunda', and d) 'Van Gogh' harvested at different stages of tuber development (from 70 to 100 DAP) (**Study III**).

A field study conducted at different latitudes in the USA (in Alaska, Florida and Texas) reported contents of phenolic acids up to two times higher in potato tubers grown in Alaska than those from lower latitudes (Payyavula et al., 2012). Alaska is characterized by longer days and lower temperatures during summer than Texas and Florida. Moreover, drought stress reduced the content of chlorogenic acid in different potato cultivars but increased the content of some minor phenolic acids such as caffeic acid (Andre et al., 2009). Our results are in agreement with these findings. Thus, high temperatures and drought stress decrease the content of phenolic acids in potato tubers.

5.4.3.4 Effect of latitude and year on the content of ascorbic acid

On average, the cultivars that grew at 61.2° N in 2014 (Kokemäki) produced tubers with the lowest contents of L-ascorbic acid, dehydroascorbic acid, and total ascorbic acid, while there were not clear differences in L-ascorbic acid in the tubers harvested in other field trials. However, the content of dehydroascorbic acid tended to be higher in the tubers at 61.2° N in 2015 (Köyliö) (**Fig. 26**).

As mentioned before, the potatoes from Kokemäki were exposed to the highest temperatures during tuber bulking and the lowest precipitation events in the early stages of tuber development. There is scarce information about the effect of weather conditions on the content of ascorbic acid in potato tubers. A field study showed that potatoes grown at warmer conditions (16.3 °C) had higher content of ascorbic acid than the cultivars grown at cooler conditions (14.7 °C). However, the weather conditions changed significantly in the three years of investigation (Hamouz et al., 2018). A study performed in Peru with 25 potato cultivars reported that potatoes grown at 3280 m above sea level had on average a higher content of ascorbic acid than the cultivars grown at higher altitudes (3700 to 3800 m above sea level) (Burgos et al., 2009). Higher altitude areas tend to be cooler than the lower regions. Thus, it can be suggested that warmer conditions increased the content of ascorbic acid. However, tubers from the lower altitude area were harvested at 150 DAP while the samples in the other areas were collected at 180 DAP which may have affected the results.

5.4.3.5 Effect of latitude and year on the gene-gene, gene-anthocyanins and anthocyanin-anthocyanin correlations

The positive gene-gene correlations in the purple-fleshed cultivars were significant in all the field trials. In the same way the positive anthocyanin-anthocyanin correlations were significant in all the field trials.

The purple-fleshed potato tubers grown at 61.2° N in 2015 (Köyliö) produced the highest positive and significant gene-anthocyanin correlations, while the purple-fleshed potato tubers that grew at 61.2° N in 2014 (Kokemäki) produced weaker and not significant positive gene-anthocyanin correlations (**Fig. 33**).

Likewise, the purple-fleshed potato tubers that grew at 64.8° N in 2015 (Muhos) produced more positive and significant correlations than the tubers from 64.8° N in 2014 (Muhos). The difference was even greater in 'Synkeä Sakari' tubers that did not produce any significant positive gene-anthocyanin correlation when grown at 64.8° N in 2014 (Muhos) (**Fig. 34**).

In the case of the red-skinned cultivar, as in the purple-fleshed cultivars, stronger positive and significant gene-gene correlations were observed for the tubers from 61.2° N in 2015 (Köyliö) than tubers that grew in the other field trials. The positive correlation between genes and anthocyanins was also stronger in the samples collected in Köyliö, especially with the main anthocyanin in this cultivar (A13: pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside), whereas there were no significant positive correlations between genes and anthocyanins in the other field trials. On the other hand, positive anthocyanin-anthocyanin correlations in 'Rosamunda' were weaker in all field trials than the anthocyanin-anthocyanin correlations found in the purple-fleshed cultivars ('Blue Congo' and 'Synkeä Sakari') (**Fig. 33**, **Fig. 34**).

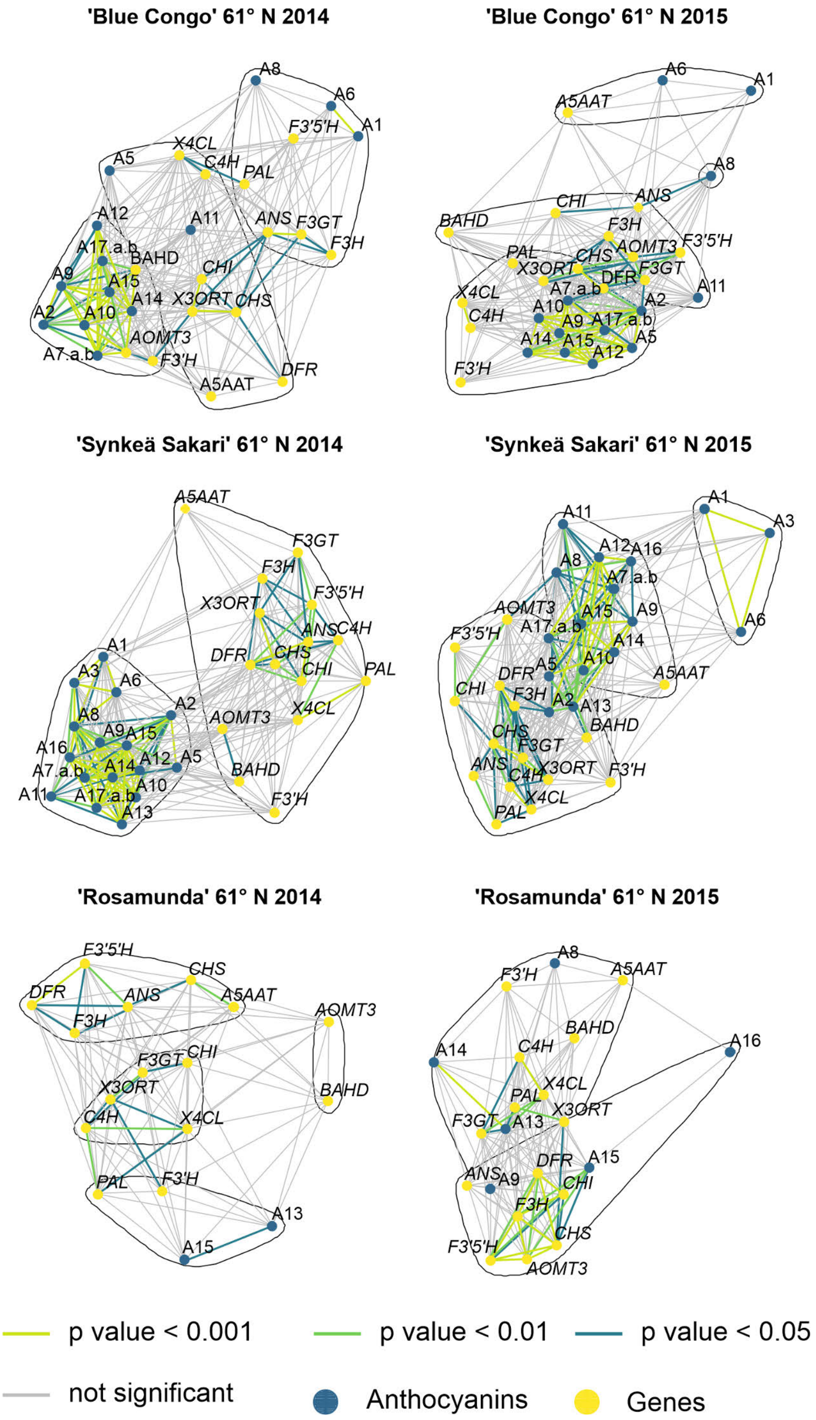


Fig. 33 Network correlation between genes and metabolites in the tubers of potato cultivars grown at 61.2° N in 2014 and 2015 (**Study III**).

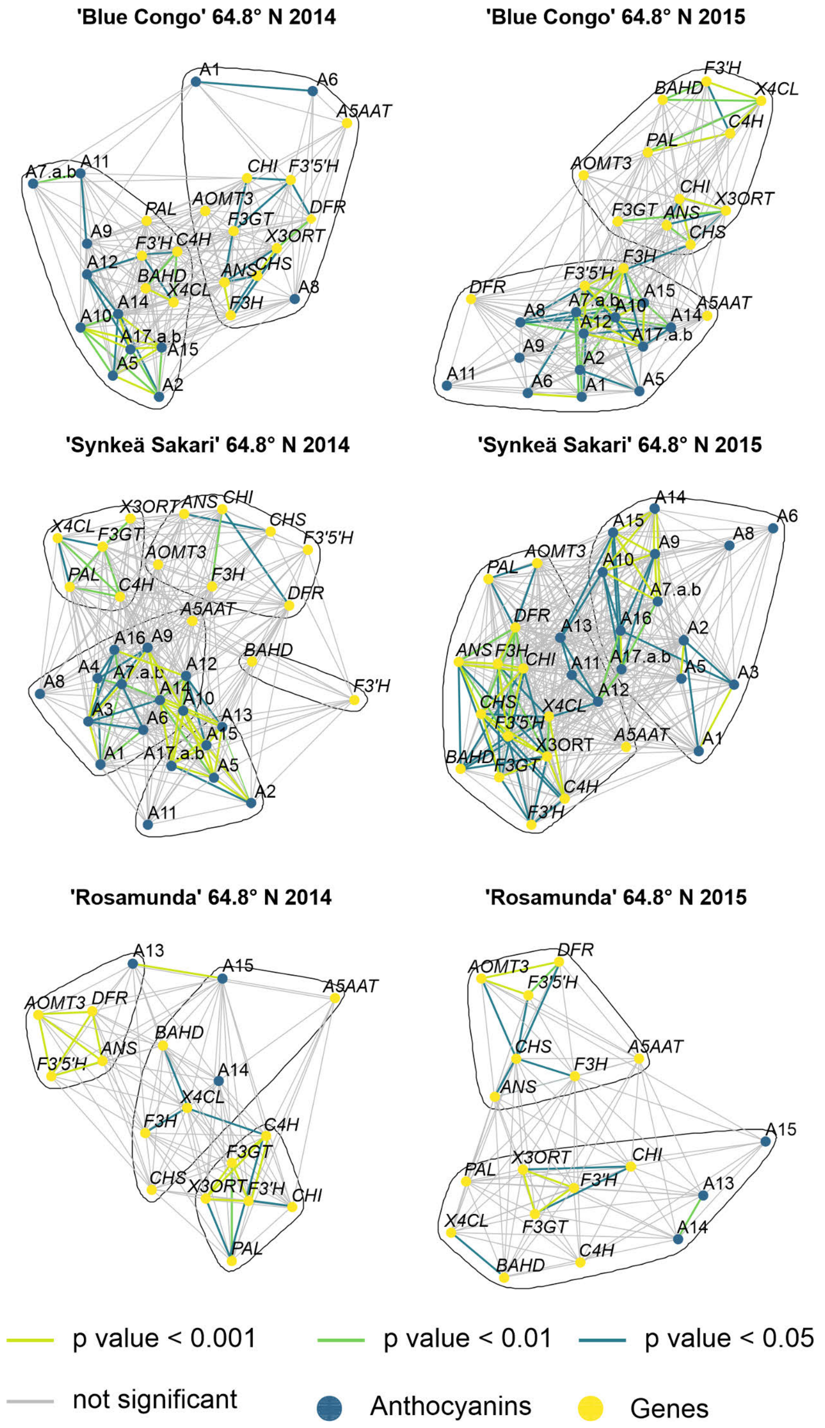


Fig. 34 Network correlation between genes and metabolites in the tubers of potato cultivars grown at 64.8° N in 2014 and 2015 (**Study III**).

6 SUMMARY AND CONCLUSION

The study showed that it is important to optimize the sample pre-treatment for extraction of anthocyanins. Freeze-dried potato samples extracted with 70 % acidified methanol gave the highest extraction yields. Additionally, steaming is an economical option for extracting anthocyanins from potatoes when freeze-drying is not possible. Steaming showed 15 % loss of anthocyanins compared to the raw-freeze dried potato samples. Determination of ascorbic acid in steamed potato samples is a better option than using raw samples. Furthermore, extraction of ascorbic acid in raw freeze-dried potatoes with TCEP, but not DTT, as reducing agent gave the highest extraction yield of ascorbic acid.

Genotype or cultivar was the main factor affecting the expression levels of the genes involved in the phenylpropanoid pathway, as well as the content of anthocyanins, phenolic acids and ascorbic acid. Purple-fleshed cultivars had higher gene expression levels than red-skinned cultivars. The main anthocyanin in the purple-fleshed cultivars was petunidin-3-*p*-coumaroylrutinoside-5-glucoside (‘Blue Congo’ and ‘Synkeä Sakari’) or malvidin-3-*p*-coumaroylrutinoside-5-glucoside (‘Blaue Veltlin’). Again, the main anthocyanin in the red-skinned cultivar (‘Rosamunda’) was pelargonidin-3-*p*-coumaroylrutinoside-5-glucoside. Cultivars with purple flesh were richer in phenolic acids than those with red or yellow flesh. The content of ascorbic acid differed between cultivars independently from the color of the flesh or skin. ‘Synkeä Sakari’ tended to produce slightly more ascorbic acid than the other cultivars.

The stage of tuber development was the second factor affecting the content of the anthocyanins and phenolic acids. The main anthocyanins and phenolic acids decreased significantly during tuber bulking. However, minor anthocyanins and phenolic acids showed oscillating values without a clear trend to increase or decrease.

The selected controlled temperatures (13 vs. 18 °C) did not significantly affect the contents of anthocyanins, phenolic acids or ascorbic acid in fully matured tubers. Thus, the selected temperature range did not induce stress conditions in the potato plants that could alter the biosynthesis of the bioactive compounds studied.

When potato cultivars were grown at different latitudes and years, the varying environmental conditions affected the expression levels of the genes involved in the phenylpropanoid pathway, and the profile of phenolic compounds, and the content of ascorbic acid. Potato plants exposed to temperatures above 18 °C and to low precipitation events produced tubers with the lowest expression levels of the genes involved in the phenylpropanoid pathway and the lowest contents of anthocyanins, phenolic acids and ascorbic acid. Potato plants exposed to temperatures between 13 and 18 °C and to good precipitation events produced tubers with the highest contents of phenolic compounds. Potato plants exposed to the lowest temperatures and global radiation showed a delayed synthesis of phenolic compounds and ascorbic acid.

Thus, the main variables affecting the synthesis of phenolic compounds and ascorbic acid were first temperature followed by precipitation and global radiation. Furthermore, the interaction effect between temperature and precipitation or between temperature and global radiation seems to strongly alter the regulation of phenolic compounds and ascorbic acid in potato tubers.

Since high temperatures and low precipitation will become common weather events due to climate change, it will be necessary to further evaluate the effects of the interaction of these weather variables when plants are exposed to short- or long-term stress conditions in different cultivars and at different stages of potato plant development to identify the cultivars most resistant to these stress conditions.

ACKNOWLEDGEMENTS

The work of this thesis was mainly performed at the Food Chemistry and Food Development unit of the Department of Life Technologies (previously Department of Biochemistry) of the University of Turku. The field cultivation trials in Muhos, Kokemäki and Köyliö (Study III) were performed with the support of LUKE - Natural Resources Institute Finland and PETLA - Potato Research Institute.

This project would not have been possible without the support of Professor Baoru Yang, PhD Anssi Vuorinen, Professor Emeritus Heikki Kallio and Professor Ritva Repo-Carrasco. Thank you for giving me the opportunity to conduct my PhD in the University of Turku. Thank you for your guidance, expertise, advice, encouragement, and patience. Professor Baoru Yang thank you for encouraging me to continue with my research project, thank you for your advice as a teacher and friend. PhD Anssi Vuorinen, thank you for having designed and organized the cultivation trials so well, without that there would be no results, and thank you for your support so that I can complete this work. Professor Emeritus Heikki Kallio thank you very much for your observations on my work, your scientific advice and suggestions always improved my work. Professor Ritva Repo-Carrasco thank you for giving me the opportunity to come to Finland before starting my PhD studies, thanks to that I could meet my supervisors in the University of Turku and had the opportunity to obtain practical experience in HPLC analysis in LUKE - Natural Resources Institute Finland (previously MTT).

I thank the financial support provided by the Center of International Mobility-Finland, Turku University Foundation, The Finnish Food Research Foundation, IANA “Indigenous Food Innovations from the Andes—funded by the Finnish Foreign Ministry, the Finnish Cultural Foundation, August Johannes and Aino Tiura Agricultural Research Foundation, the Graduate School of University of Turku, and Food Sciences Unit, and the Department of Life Technologies of the University of Turku.

I thank PhD Mikko Griinari for providing the seeds of the cultivar ‘Synkeä Sakari’. I also thank Elina Virtanen for carrying out the cultivation trial in Muhos and for collecting the samples at the different stages of tuber development. Thanks to Anna Sipilä and Jussi Tuomisto for collaborating with the cultivation trial in Kokemäki and Köyliö.

I thank the guidance and support with instrument problems to Professor Jukka-Pekka Suomela, PhD Oskar Laaksonen, PhD Marko Tarvainen, Jani Sointusalo, and Tapio Ronkainen. Thanks to Anu Hirvensalo, Mirva Jalo and Leena Neuvonen for your administrative support.

I also would like to help the collaboration with the chemical analysis and friendship of Wenjia He, Priscilla Ollennu-Chuasam and Sreeman Barua. Special thanks to PhD Niko Markkinen for guiding the qPCR analysis.

I thank all my colleagues at the Food Chemistry and Food Development unit for your friendship, kindness, and help.

I also would like to express my gratitude to Jarkko Hellström who guided me since the initial stages of my PhD studies, advised me on how to solve HPLC problems, and is my company and friend.

I thank my parents and siblings for your example, guidance, strength, and constant communication. I thank my father who was also my professor during my bachelor studies, for his guidance as a chemical engineer and as an environmental chemist, for his suggestions on how to improve my research, for giving me strength to face life and for showing me the beauty of life.

Turku, December 2023

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APPENDIX: ORIGINAL PUBLICATIONS

I Reprinted from Food Chemistry 2018, 242, 217–224, with permission from Elsevier.

II Reprinted from Food Chemistry 2020, 326, 126966, with permission from Elsevier.

III Submitted manuscript.

DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

1. **REINO R. LINKO (1967)** Fatty acids and other components of Baltic herring flesh lipids. (Organic chemistry).
2. **HEIKKI KALLIO (1975)** Identification of volatile aroma compounds in arctic bramble, *Rubus arcticus* L. and their development during ripening of the berry, with special reference to *Rubus stellatus* SM.
3. **JUKKA KAITARANTA (1981)** Fish roe lipids and lipid hydrolysis in processed roe of certain *Salmonidae* fish as studied by novel chromatographic techniques.
4. **TIMO HIRVI (1983)** Aromas of some strawberry and blueberry species and varieties studied by gas liquid chromatographic and selected ion monitoring techniques.
5. **RAINER HUOPALAHTI (1985)** Composition and content of aroma compounds in the dill herb, *Anethum graveolens* L., affected by different factors.
6. **MARKKU HONKAVAARA (1989)** Effect of porcine stress on the development of PSE meat, its characteristics and influence on the economics of meat products manufacture.
7. **PÄIVI LAAKSO (1992)** Triacylglycerols – approaching the molecular composition of natural mixtures.
8. **MERJA LEINO (1993)** Application of the headspace gas chromatography complemented with sensory evaluation to analysis of various foods.
9. **KAISLI KERROLA (1994)** Essential oils from herbs and spices: isolation by carbon dioxide extraction and characterization by gas chromatography and sensory evaluation.
10. **ANJA LAPVETELÄINEN (1994)** Barley and oat protein products from wet processes: food use potential.
11. **RAIJA TAHVONEN (1995)** Contents of lead and cadmium in foods in Finland.
12. **MAIJA SAXELIN (1995)** Development of dietary probiotics: estimation of optimal *Lactobacillus* GG concentrations.
13. **PIRJO-LIISA PENTTILÄ (1995)** Estimation of food additive and pesticide intakes by means of a stepwise method.
14. **SIRKKA PLAAMI (1996)** Contents of dietary fiber and inositol phosphates in some foods consumed in Finland.
15. **SUSANNA EEROLA (1997)** Biologically active amines: analytics, occurrence and formation in dry sausages.
16. **PEKKA MANNINEN (1997)** Utilization of supercritical carbon dioxide in the analysis of triacylglycerols and isolation of berry oils.
17. **TUULA VESA (1997)** Symptoms of lactose intolerance: influence of milk composition, gastric emptying, and irritable bowel syndrome.
18. **EILA JÄRVENPÄÄ (1998)** Strategies for supercritical fluid extraction of analytes in trace amounts from food matrices.
19. **ELINA TUOMOLA (1999)** *In vitro* adhesion of probiotic lactic acid bacteria.
20. **ANU JOHANSSON (1999)** Availability of seed oils from Finnish berries with special reference to compositional, geographical and nutritional aspects.
21. **ANNE PIHLANTO-LEPPÄLÄ (1999)** Isolation and characteristics of milk-derived bioactive peptides.
22. **MIKA TUOMOLA (2000)** New methods for the measurement of androstenone and skatole – compounds associated with boar taint problem. (Biotechnology).
23. **LEEA PELTO (2000)** Milk hypersensitivity in adults: studies on diagnosis, prevalence and nutritional management.
24. **ANNE NYKÄNEN (2001)** Use of nisin and lactic acid/lactate to improve the microbial and sensory quality of rainbow trout products.
25. **BAORU YANG (2001)** Lipophilic components of sea buckthorn (*Hippophaë rhamnoides*) seeds and berries and physiological effects of sea buckthorn oils.
26. **MINNA KAHALA (2001)** Lactobacillar S-layers: Use of *Lactobacillus brevis* S-layer signals for heterologous protein production.
27. **OLLI SJÖVALL (2002)** Chromatographic and mass spectrometric analysis of non-volatile oxidation products of triacylglycerols with emphasis on core aldehydes.
28. **JUHA-PEKKA KURVINEN (2002)** Automatic data processing as an aid to mass spectrometry of dietary triacylglycerols and tissue glycerophospholipids.
29. **MARI HAKALA (2002)** Factors affecting the internal quality of strawberry (*Fragaria x ananassa* Duch.) fruit.
30. **PIRKKA KIRJAVAINEN (2003)** The intestinal microbiota – a target for treatment in infant atopic eczema?
31. **TARJA ARO (2003)** Chemical composition of Baltic herring: effects of processing and storage on fatty acids, mineral elements and volatile compounds.
32. **SAMI NIKOSKELAINEN (2003)** Innate immunity of rainbow trout: effects of opsonins, temperature and probiotics on phagocytic and complement activity as well as on disease resistance.
33. **KAISA YLI-JOKIPII (2004)** Effect of triacylglycerol fatty acid positional distribution on postprandial lipid metabolism.
34. **MARIKA JESTOI (2005)** Emerging *Fusarium*-mycotoxins in Finland.
35. **KATJA TIITINEN (2006)** Factors contributing to sea buckthorn (*Hippophaë rhamnoides* L.) flavour.

36. **SATU VESTERLUND (2006)** Methods to determine the safety and influence of probiotics on the adherence and viability of pathogens.
37. **FANDI FAWAZ ALI IBRAHIM (2006)** Lactic acid bacteria: an approach for heavy metal detoxification.
38. **JUKKA-PEKKA SUOMELA (2006)** Effects of dietary fat oxidation products and flavonols on lipoprotein oxidation.
39. **SAMPO LAHTINEN (2007)** New insights into the viability of probiotic bacteria.
40. **SASKA TUOMASJUKKA (2007)** Strategies for reducing postprandial triacylglycerolemia.
41. **HARRI MÄKIVUOKKO (2007)** Simulating the human colon microbiota: studies on polydextrose, lactose and cocoa mass.
42. **RENATA ADAMI (2007)** Micronization of pharmaceuticals and food ingredients using supercritical fluid techniques.
43. **TEEMU HALTTUNEN (2008)** Removal of cadmium, lead and arsenic from water by lactic acid bacteria.
44. **SUSANNA ROKKA (2008)** Bovine colostral antibodies and selected lactobacilli as means to control gastrointestinal infections.
45. **ANU LÄHTEENMÄKI-UUTELA (2009)** Foodstuffs and medicines as legal categories in the EU and China. Functional foods as a borderline case. (Law).
46. **TARJA SUOMALAINEN (2009)** Characterizing *Propionibacterium freudenreichii* ssp. *shermanii* JS and *Lactobacillus rhamnosus* LC705 as a new probiotic combination: basic properties of JS and pilot *in vivo* assessment of the combination.
47. **HEIDI LESKINEN (2010)** Positional distribution of fatty acids in plant triacylglycerols: contributing factors and chromatographic/mass spectrometric analysis.
48. **TERHI POHJANHEIMO (2010)** Sensory and non-sensory factors behind the liking and choice of healthy food products.
49. **RIIKKA JÄRVINEN (2010)** Cuticular and suberin polymers of edible plants – analysis by gas chromatographic-mass spectrometric and solid state spectroscopic methods.
50. **HENNA-MARIA LEHTONEN (2010)** Berry polyphenol absorption and the effect of northern berries on metabolism, ectopic fat accumulation, and associated diseases.
51. **PASI KANKAANPÄÄ (2010)** Interactions between polyunsaturated fatty acids and probiotics.
52. **PETRA LARMO (2011)** The health effects of sea buckthorn berries and oil.
53. **HENNA RÖYTIÖ (2011)** Identifying and characterizing new ingredients *in vitro* for prebiotic and synbiotic use.
54. **RITVA REPO-CARRASCO-VALENCIA (2011)** Andean indigenous food crops: nutritional value and bioactive compounds.
55. **OSKAR LAAKSONEN (2011)** Astringent food compounds and their interactions with taste properties.
56. **ŁUKASZ MARCIN GRZEŚKOWIAK (2012)** Gut microbiota in early infancy: effect of environment, diet and probiotics.
57. **PENGZHAN LIU (2012)** Composition of hawthorn (*Crataegus* spp.) fruits and leaves and emblic leafflower (*Phyllanthus emblica*) fruits.
58. **HEIKKI ARO (2012)** Fractionation of hen egg and oat lipids with supercritical fluids. Chemical and functional properties of fractions.
59. **SOILI ALANNE (2012)** An infant with food allergy and eczema in the family – the mental and economic burden of caring.
60. **MARKO TARVAINEN (2013)** Analysis of lipid oxidation during digestion by liquid chromatography-mass spectrometric and nuclear magnetic resonance spectroscopic techniques.
61. **JIE ZHENG (2013)** Sugars, acids and phenolic compounds in currants and sea buckthorn in relation to the effects of environmental factors.
62. **SARI MÄKINEN (2014)** Production, isolation and characterization of bioactive peptides with antihypertensive properties from potato and rapeseed proteins.
63. **MIKA KAIMAINEN (2014)** Stability of natural colorants of plant origin.
64. **LOTTA NYLUND (2015)** Early life intestinal microbiota in health and in atopic eczema.
65. **JAAKKO HIIDENHOVI (2015)** Isolation and characterization of ovomucin – a bioactive agent of egg white.
66. **HANNA-LEENA HIETARANTA-LUOMA (2016)** Promoting healthy lifestyles with personalized, *APOE* genotype based health information: The effects on psychological-, health behavioral and clinical factors.
67. **VELI HIETANIEMI (2016)** The *Fusarium* mycotoxins in Finnish cereal grains: How to control and manage the risk.
68. **MAARIA KORTESNIEMI (2016)** NMR metabolomics of foods – Investigating the influence of origin on sea buckthorn berries, *Brassica* oilseeds and honey.
69. **JUHANI AAKKO (2016)** New insights into human gut microbiota development in early infancy: influence of diet, environment and mother's microbiota.
70. **WEI YANG (2017)** Effects of genetic and environmental factors on proanthocyanidins in sea buckthorn (*Hippophaë rhamnoides*) and flavonol glycosides in leaves of currants (*Ribes* spp.).
71. **LEENAMAIJA MÄKILÄ (2017)** Effect of processing technologies on phenolic compounds in berry products.

72. **JUHA-MATTI PIHLAVA (2017)** Selected bioactive compounds in cereals and cereal products – their role and analysis by chromatographic methods.
73. **TOMMI KUMPULAINEN (2018)** The complexity of freshness and locality in a food consumption context
74. **XUEYING MA (2018)** Non-volatile bioactive and sensory compounds in berries and leaves of sea buckthorn (*Hippophaë rhamnoides*)
75. **ANU NUORA (2018)** Postprandial lipid metabolism resulting from heated beef, homogenized milk and interesterified palm oil.
76. **HEIKKI AISALA (2019)** Sensory properties and underlying chemistry of Finnish edible wild mushrooms.
77. **YE TIAN (2019)** Phenolic compounds from Finnish berry species to enhance food safety.
78. **MAIJA PAAKKI (2020)** The importance of natural colors in food for the visual attractiveness of everyday lunch.
79. **SHUXUN LIU (2020**) Fermentation with non-*Saccharomyces* yeasts as a novel biotechnology for berry wine production.
80. **MARIKA KALPIO (2020)** Strategies for analyzing the regio- and stereospecific structures of individual triacylglycerols in natural fats and oils.
81. **JOHANNA JOKIOJA (2020)** Postprandial effects and metabolism of acylated anthocyanins originating from purple potatoes.
82. **NIINA KELANNE (2021)** Novel bioprocessing for increasing consumption of Nordic berries.
83. **NIKO MARKKINEN (2021)** Bioprocessing of berry materials with malolactic fermentation.
84. **GABRIELE BELTRAME (2021)** Polysaccharides from Finnish fungal resources.
85. **SALLA LAITO (2022)** Bioactive compounds in oats and gut health.
86. **KANG CHEN (2022)** Multi-omics study on the effects of anthocyanin extracts from bilberries and purple potatoes on type 2 diabetes in Zucker diabetic fatty rats.
87. **WENJIA HE (2022)** Bioprocessing of alcoholic beverages from apples and pears: Effects of raw materials and processes on quality.
88. **TANJA KAKKO (2023)** Alternative approaches to improve the processing and quality of under-utilized fish.
89. **MIKAEL FABRITIUS (2023)** Mass spectrometric methodologies for analysis of triacylglycerol and phospholipid regioisomers in natural fats and oils.
90. **ELLA AITTA (2023)** Green technologies for the extraction of oil and protein from Baltic herring (*Clupea harengus membras*).
91. **AMRUTA KULKARNI (2023)** Effect of omega-3 deficiency and positional distribution of docosahexaenoic acid in triacylglycerols on tissue lipids in rats.
92. **LIZ A. GUTIÉRREZ QUEQUEZANA (2023)** Effect of cultivar, growth environment and developmental stage on phenolic compounds and ascorbic acid in potato tubers grown in Finland.

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