DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

Postprandial Effects and Metabolism of Acylated Anthocyanins Originating from Purple Potatoes

JOHANNA JOKIOJA

Food Chemistry and Food Development
Department of Biochemistry

DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU
Food Chemistry

Postprandial Effects and Metabolism of Acylated Anthocyanins Originating from Purple Potatoes

JOHANNA JOKIOJA

Food Chemistry and Food Development
Department of Biochemistry

TURKU, FINLAND – 2020

Food Chemistry and Food Development
Department of Biochemistry
University of Turku, Finland

Supervised by

Professor Baoru Yang, Ph.D.
Department of Biochemistry
University of Turku
Turku, Finland

Associate Professor Kaisa Linderborg, Ph.D.
Department of Biochemistry
University of Turku
Turku, Finland

Reviewed by

Professor Emerita Kaisa Poutanen, Ph.D.
VTT Technical Research Centre of Finland Ltd
Espoo, Finland

Ana Rodriguez-Mateos, Ph.D.
Department of Nutritional Sciences
King's College London
London, United Kingdom

Opponent

Professor Fulvio Mattivi, Ph.D.
Department of Cellular, Computational, and Integrative Biology-CIBIO
University of Trento
San Michele all'Adige, Italy

Research director

Professor Baoru Yang, Ph.D.
Department of Biochemistry
University of Turku
Turku, Finland

The originality of this dissertation has been checked in accordance with the University of Turku quality assurance system using the Turnitin OriginalityCheck service

ISBN 978-951-29-8291-2 (print)
ISBN 978-951-29-8292-9 (pdf)
ISSN 2323-9395 (print)
ISSN 2323-9409 (pdf)
Painosalama Oy – Turku, Finland 2020

TABLE OF CONTENTS

ABSTRACT

Healthy dietary choices, such as daily consumption of vegetables and fruits, are fundamental for preventing metabolic disorders. Anthocyanin-rich purple and red edible plants, such as berries and fruits, show potential in improving carbohydrate metabolism. The large diversity of structural characteristics of anthocyanins, such as the number and position of hydroxy, methoxy, glycosyl and acyl substituents, affect their physico-chemical properties and followingly, their absorption and metabolism. However, the health outcomes and metabolism of anthocyanins have been studied mainly with foods rich in non-acylated anthocyanins. Acylated anthocyanins, instead, provide enhanced structural stability for the otherwise unstable anthocyanins and thus have the potential to increase the use of anthocyanins in food industrial applications. Acylated anthocyanins are commonly found in pigmented vegetables and tubers, such as potatoes, which may provide an affordable dietary source of anthocyanins that are easily incorporated into an everyday diet for increased intake of anthocyanins.

The aim of the current work was to investigate the *in vivo* effect of acylated anthocyanins on postprandial glycaemia, insulinaemia and inflammation, and to investigate the metabolism of acylated anthocyanins in healthy volunteers. A pigmented potato variety (*Solanum tuberosum* L. ′Synkeä Sakari′) with purple skin and flesh was chosen as the model food for these studies since it has a high content of acylated anthocyanins rich in methoxysubstituted B-rings. Furthermore, potatoes are widely consumed and cultivated worldwide and have a high glycaemic index. In two cross-over clinical trials, healthy volunteers consumed a study meal of cooked and mashed purple potatoes or their extract rich in acylated anthocyanins mixed with yellow potatoes, with cooked and mashed yellow potatoes as the control meal. The meals were characterised using high-performance liquid chromatography (HPLC), gas chromatography (GC) and mass spectrometry (MS). The plasma glucose, insulin and 90 inflammation markers were measured. The phenolic metabolites and their phase II conjugates from serially collected plasma and urine samples were screened using UHPLC-MS/MS.

Both the purple potatoes and the anthocyanin-rich purple potato extract decreased the highest peak of postprandial blood glucose levels as compared to a meal of yellow potatoes. The anthocyanin-rich extract prevented glucose and insulin levels of plasma from decreasing below their fasting state levels, which occurred with the yellow potato meal. Additionally, the anthocyanin-rich extract altered the inflammation markers; for example, the insulin-like FGF-19 was increased four hours after the meal compared to the control meal. These observed health effects may have been affected by postprandial degradants of the ingested

acylated anthocyanins, as the parent compounds were not detected in the plasma or urine samples. Instead, a large diversity of phenolic metabolites, such as hydroxycinnamic and hydroxybenzoic acids, were detected. Furthermore, the degradants were subjected to conjugation in phase II metabolism. Several metabolites previously not detected after a meal rich in acylated anthocyanins were identified, such as protocatechuic acid sulfates and glucuronides.

This work provides evidence that purple potatoes and their extract rich in acylated anthocyanins affect postprandial carbohydrate metabolism and inflammation beneficially after a high carbohydrate meal. The results shed light on the metabolism of acylated anthocyanins, phenolic metabolites in postprandial state, and promote the use of anthocyanin-rich purple potatoes as a part of a healthy and versatile diet.

SUOMENKIELINEN ABSTRAKTI

Terveellinen ruokavalio, kuten runsas kasvisten ja vihannesten nauttiminen, edesauttaa aineenvaihdunnan häiriöiden ennaltaehkäisemisessä. Aiemmat tutkimukset osoittavat, että violeteilla ja punaisilla antosyaniineja sisältävillä syötävillä kasveilla, kuten marjoilla ja hedelmillä, saattaa olla hiilihydraatti-aineenvaihdunnalle edullisia terveysvaikutuksia. Antosyaniinit ovat kuitenkin rakenteellisesti monipuolinen yhdisteryhmä, jonka hydroksyyli-, metoksi-, sokeri- ja asyylisubstituentit vaikuttavat niiden fysikokemiallisiin ominaisuuksiin ja siten niiden imeytymiseen ja aineenvaihduntaan. Asyloitujen antosyaniinien vaikutusta ihmisen aterianjälkeiseen aineenvaihduntaan on tutkittu vain vähän, vaikka ne soveltuvat asyloimattomia antosyaniineja pysyvämpinä yhdisteinä paremmin elintarviketeollisuuden tuoteinnovaatioihin. Asyloituja antosyaniineja on runsaasti värillissä juureksissa ja mukuloissa, kuten perunoissa, joita hyödyntämällä on mahdollisuus lisätä antosyaniinien saantia edullisesti ja yksinkertaisesti osana päivittäistä ruokavaliota.

Väitöskirjan kokeellisessa osassa tutkittiin asyloitujen antosyaniinien vaikutusta aterianjälkeiseen glykemiaan, insulinemiaan ja tulehdukseen sekä asyloitujen antosyaniinien pilkkoutumista terveiden ihmisten aineenvaihdunnassa. Asyloitujen antosyaniinien elintarvikelähteeksi valittiin violettimaltoinen ja -kuorinen perunalajike (*Solanum tuberosum* L. ′Synkeä Sakari′), koska sen antosyaniinit ovat valtaosin asyloituja. Lisäksi perunaa käytetään ja viljellään maailmanlaajuisesti, ja niillä on pääsääntöisesti korkea glykeeminen indeksi. Tässä työssä järjestettiin kaksi yksöissokotettua kliinistä vaihtovuorotutkimusta, joissa terveet tutkimushenkilöt nauttivat kypsennettyjä ja soseutettuja violetteja perunoita tai violettien perunoiden antosyaniinipitoista uutetta sekoitettuna soseutettuihin keltamaltoisiin perunoihin. Kontrolliateriana tutkimushenkilöille annettiin kypsennettyjä ja soseutettuja keltamaltoisia perunoita. Tutkimusaterioiden kemiallinen koostumus määritettiin käyttämällä korkean erotuskyvyn nestekromatografiaa (HPLC), kaasukromatografiaa (GC) ja massaspektrometriaa (MS). Tutkimushenkilöiltä kerätyistä plasmanäytteistä määritettiin veren glukoosi- ja insuliinipitoisuudet ja 90 tulehdusmerkkiainetta. Virtsa- ja verinäytteistä selvitettiin fenolisia aineenvaihduntatuotteita (UHPLC-MS/MS).

Sekä violetit perunat että niiden antosyaniinipitoinen uute laskivat aterianjälkeistä korkeinta glukoosi- ja insuliinihuippua keltaisiin perunoihin verrattuna. Uute esti kontrolliaterian aiheuttaman glukoosi- ja insuliinipitoisuuksien laskemisen paastotason alapuolelle. Lisäksi uute vaikutti aterianjälkeisiin tulehdusmerkkiaineisiin. Esimerkiksi insuliininkaltaisen FGF-19:n pitoisuus kasvoi neljä tuntia aterian jälkeen kontrolliateriaan verrattuna. Havaittuihin terveysvaikutuksiin saattoi vaikuttaa asyloitujen antosyaniinien

lisäksi niiden aterianjälkeiset hajoamistuotteet, koska perunan antosyaniineja ei havaittu sellaisinaan plasmasta tai virtsasta. Sen sijaan tutkimuksessa havaittiin runsaasti erilaisia fenolisia aineenvaihduntatuotteita, kuten hydroksikanelihappoja ja hydroksibentsoehappoja. Lisäksi aineenvaihduntatuotteisiin saatettiin konjugoida faasin II metaboliassa. Työssä tunnistettiin aineenvaihduntatuotteita, kuten protokatekiinihappoa ja sen konjugaatteja, joita ei oltu aiemmin havaittu pääosin asyloituja antosyaniineja sisältäneen aterian jälkeen.

Väitöskirjatutkimus antaa viitteitä siitä, että violeteilla perunoilla ja niiden asyloituja antosyaniineja sisältävällä uutteella on myönteinen vaikutus ihmisen aterianjälkeiseen aineenvaihduntaan ja tulehdukseen runsashiilihydraattisen aterian jälkeen. Tulokset kuvaavat asyloitujen antosyaniinien pilkkoutumista ja fenolisia aineenvaihduntatuotteita aterianjälkeisessä tilassa. Lisäksi tutkimustulokset kannustavat violettimaltoisten perunoiden käyttöön osana terveellistä ja monipuolista ruokavaliota.

LIST OF ABBREVIATIONS

AMPK	Adenosine monophosphate activated protein kinase
BMI	Body mass index
DAD	Diode array detector
C_{max}	Maximum concentration
ECLIA	Electrochemiluminescence immunoassay
ESI	Electrospray ionization
FID	Flame-ionization detector
FW	Fresh weight
G6P	Glucose-6-phosphatase
GC	Gas chromatography
GLUT	Glucose transporter
HbA1c	Glycated haemoglobin
HPLC	High-performance liquid chromatography
iAUC	Incremental area under the curve
ipGTT	Intraperitoneal glucose tolerance test
ipITT	Intraperitoneal insulin tolerance test
MCT1	Monocarboxylated transporter 1
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
m/z	Mass-to-charge ratio
OGTT	Oral glucose tolerance test
PEPCK	Phosphoenolpyruvate carboxykinase
PPE	Purple potato extract
SGLT1	Sodium-glucose cotransporter 1
SPE	Solid-phase extraction
TFA	Trifluoroacetic acid
t_{max}	Time at maximum concentration
UHPLC	Ultra-high performance liquid chromatography

LIST OF ORIGINAL PUBLICATIONS

I. Linderborg, K.; Salo* J.; Kalpio M.; Vuorinen A.; Kortesniemi M.; Griinari M.; Viitanen M.; Yang B.; Kallio H. Comparison of the postprandial effects of purple-fleshed and yellow-fleshed potatoes in healthy males with chemical characterization of the potato meals. *International Journal of Food Sciences and Nutrition*, **2016**, *67* (5), 581–591.

II. Jokioja, J.; Linderborg, K.; Kortesniemi, M.; Nuora, A.; Heinonen, J.; Sainio, T.; Viitanen, M.; Kallio, H.; Yang, B. Anthocyanin-rich extract from purple potatoes decreases postprandial glycemic response and affects inflammation markers in healthy men. *Food Chemistry*, **2020**, *310*, 125797.

III. Jokioja, J.; Percival, J; Philo, M.; Yang, B.; Kroon, P.; Linderborg K. Phenolic metabolites in the urine and plasma of healthy men after acute intake of purple potato extract rich in methoxysubstituted monoacylated anthocyanins. *Submitted manuscript.*

*Previous surname (Johanna Jokioja, née Salo)

1 INTRODUCTION

Intake of food is followed by complex and dynamic metabolic processes involving various endocrine signals, enzymes, biochemical reactions and almost all organs and tissues, all aimed at exploiting the macro- and micronutrients of food for survival and maintaining homeostasis. This metabolic state of nutrient digestion and absorption is called the postprandial state.[1] Health, on the other hand, has been defined by the World Health Organization in 1948 as a state of complete physical, mental and social well-being and not merely as the absence of disease or infirmity.[2] Health and the postprandial state are profoundly linked as an imbalance in the intake of nutrients impairs the processes of postprandial metabolism leading to oxidative stress, chronic systemic low-grade inflammation, and finally, to severe metabolic disorders such as type II diabetes and metabolic syndrome.[1,3] Repetitive, oscillating high blood glucose peaks especially are more detrimental to health than constantly elevated baseline glucose levels (hyperglycaemia).[4] The importance of the postprandial state on human health is emphasised by its duration: it may cover the majority of a day, as cumulative periods of 4–6 hours after food consumption.[5]

Dietary choices, such as daily consumption of vegetables and fruits, is part of the strategy for preventing metabolic disorders in addition to other lifestyle changes.[6–8] The emerging research on the red and purple polyphenolic colours of vegetables, fruits, and berries, the anthocyanins, has shown clinical potential in promoting postprandial carbohydrate metabolism.[9–13] Currently, the most popular anthocyanin sources in Europe are red wine, fruits, berries and some leafy vegetables, whereas berries and berry products dominate in Finland and Australia.[14–16] Estimates of the daily intake of anthocyanins vary greatly depending on the country, age and gender; adult Europeans consume from 18.7 mg to 64.9 mg of anthocyanins daily[14], Australians 32.9 mg[17] and US Americans 11.6 mg[18], whereas the intake of anthocyanins in German children is only 6.0 mg[19]. Anthocyanins are not recognised as an essential part of nutrition, and currently, only China has set a recommended daily intake of 50 mg for anthocyanins.[20]

However, anthocyanins are structurally unstable, which compromises their industrial usage in food as they are easily degraded during food processing and storage. Interestingly, substitution with acyl groups stabilises anthocyanins[21] and enhances the colour strength[22] compared to non-acylated anthocyanins, and therefore acylation offers the food industry new solutions, such as more stable natural food colourants. Acylated anthocyanins are found in pigmented vegetables and root vegetables and in minor amounts in some berries and fruits.[23,24] Despite the potential of acylated anthocyanins, the clinical trials investigating the effect of anthocyanins on postprandial carbohydrate

metabolism have been conducted mainly with berries and fruits rich in non-acylated ones.[9–13]

Bioavailability of anthocyanins, *i.e.* the amount of the ingested anthocyanins absorbed and thus available for physiological use, is notoriously poor. A majority of studies show that the urinary recovery of anthocyanins is well below 1%.[25–27] Acylation decreases the bioavailability even further; for example, the bioavailability of acylated anthocyanins of purple carrots and red cabbage is 4–14 times less in comparison to non-acylated anthocyanins.[28–30] Therefore, the phenolic metabolites of ingested anthocyanins may contribute to the observed health effects of the poorly absorbed parent compounds.[26,31,32] As the metabolism studies have also focused mainly on berries and fruits rich in non-acylated anthocyanins[32–40], there is a clear need to investigate the metabolites of acylated anthocyanins in addition to their health effects.

The aim of the experimental research part of this thesis was to study the effect of dietary acylated anthocyanins on human postprandial glycaemia, insulinaemia and inflammation, and to investigate the metabolism of acylated anthocyanins. The model food chosen for the clinical trials was purple potatoes which contain anthocyanins mostly in monoacylated form (98%)[41] and have high glycaemic index[42,43]. The particular variety, *Solanum tuberosum* L. ′Synkeä Sakari′, has purple anthocyanin-rich skin and flesh. The content of anthocyanins in pigmented potatoes is variety-specific[44–46], typically ranging between 0.7–74.3 mg /100 g fresh weight[45], the average being 41.3 mg/100 g FW[44]. Purple potatoes, as a common everyday source, have the potential to increase the daily dietary intake of anthocyanins, as potatoes are already the most cultivated non-grain staple crop consumed all over the world; they also have excellent nutritive value as they are rich in energy, carbohydrates, potassium, vitamin C and B6, but low in fat.[44] Due to their high anthocyanin content[47], purple potatoes have a double-triple increase in antioxidant activity as compared to the white potatoes[48], thus introducing potential food industry applications.[23,49] Considering sustainability, potatoes generate low environmental burden due to their domestic production, low requirement for water and low greenhouse gas emissions.[50]

This literature review discusses the interplay between anthocyanins, postprandial glucose metabolism and inflammation, on the grounds of current literature with special emphasis on acylated anthocyanins and purple-fleshed potatoes when possible. The experimental research work conducted for this thesis focuses on the effect of purple potatoes and their extracts rich in acylated anthocyanins on acute postprandial carbohydrate metabolism and inflammation markers of healthy male volunteers with a thorough screening of possibly health-promoting phenolic metabolites. Overall, this thesis strives to discover new findings to increase the current understanding of the postprandial health effects and metabolism of acylated anthocyanins.

2 REVIEW OF THE LITERATURE

2.1 Pigmented potatoes as a source of anthocyanins

2.1.1 Pigmented potatoes

Potatoes (*Solanum* spp.), of the family of Solanaceae, are herbaceous plants which form edible starchy tubers as their primary storage organs at the terminal ends of their underground lateral stems called stolons.[51] They have a long history of cultivation stretching back over thousands of years to the Andes when they were domesticated from a monophyletic *Solanum brevicaule* complex.[52] The taxonomy of potatoes is controversial, with recent estimates of 107 wild and four cultivated species (*Solanum tuberosum, S. juzepczukii, S. ajanhuiri and S. curtilobum*).[53] The ploidy level of potatoes varies from diploid up to pentaploids (cultivated species) and hexaploids (wild species).[51,53]

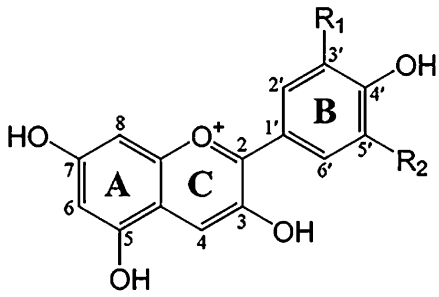
The focus of this thesis is on the pigmented anthocyanin-rich varieties of the common potato *Solanum tuberosum* L. cultivated and consumed all over the world.[53] The pigmented varieties have distinguishable morphological traits due to the differences in genetics giving them a pigmented phenotype in the tuber flesh, skin and flower.[54] Depending on the variety, the flesh of the pigmented tubers may be partially or totally pigmented with light or dark red or purple, or even white/yellow, whereas the skin is pigmented.[55–59] Also the shape of the tubers may vary from round to oval, oblong and fingerling.[57,59] Many pigmented potato cultivars are known, such as the purple cultivars Blue Congo, Salad Blue, Shetland Black, Violette and Vitelotte, and the red cultivars Highland Burgundy Red and Salad Red.[45] An example of a purple variety, *Solanum tuberosum* L. ′Synkeä Sakari′ which was used in the experimental part of this thesis, is shown in **Figure 1**.

The pigmented potato varieties have varying amounts of anthocyanins, with the average content of 41.3 mg/100 g FW.[44] For example, Blue Congo contains 0.7–18.6 mg/100 g FW of anthocyanins, whereas Vitelotte contains 30.7–64.2 and Highland Burgundy Red 2.9–23.9 mg/100 g FW of anthocyanins when cultivated in two different locations in the Czech Republic.[45] The amount of anthocyanins is affected by several genetic, environmental and developmental factors which are not all fully understood. In addition to variety, these factors include for example light and temperature.[45,60] The role of anthocyanins in plants is to contribute to the defence system against different biotic and abiotic stress,[61] and interestingly, the cold temperature and long light period during the growth season in northern locations have been detected to promote the synthesis of anthocyanins in pigmented potatoes as a response to these stress factors.[59,62]

Figure 1. Examples of the phenotypes of A) leaves and stems, B) flowers and C) whole and longitudinally cut tubers of the purple pigmented potato variety *Solanum tuberosum* L. ′Synkeä Sakari′. Photographs A and C were taken by Johanna Jokioja, and B is published with the permission of the photographer, Mikko Lindfors.

2.1.2 Acylated anthocyanins of pigmented potatoes

Anthocyanins are a subclass of flavonoids responsible for a range of pigmentation from salmon-pink to red and purple of various fruits, berries, vegetables, roots, leaves, flowers and pigmented potatoes. Like other flavonoids, anthocyanins are structured by a C6-C3-C6 ring system, which contains two hydroxysubstituted benzenes (A- and B-rings) linked by a heterocyclic ring (C-ring). In anthocyanins, this ring system is a 2-phenylbenzopyrylium cation or a flavylium cation. The flavylium cation of anthocyanins is π-conjugated, resulting in chromophoric properties. The carbons 4′, 3, 5 and 7 are hydroxylated to form the anthocyanin aglycone, anthocyanidin, and the hydroxy- and methoxysubsitutents in carbons 3′ and 5′ determine the name of the anthocyanidin. The common six anthocyanidins found in edible plants are cyanidin, delphinidin, malvidin, pelargonidin, peonidin and petunidin (**Figure 2**).[20,24,63,64] Pigmented potatoes contain all six of them.[41,65–71,57,46,72–75] The major anthocyanidins of purple potato varieties are methoxysubstituted petunidin, peonidin and malvidin[41,65–67,70], whereas the major anthocyanidin in red varieties is pelargonidin[46,66,73,75].



Anthocyanidin	R_1	R_2
Cyanidin	OH	H
Delphinidin	OH	OH
Malvidin	OCH_3	OCH_3
Pelargonidin	H	H
Peonidin	OCH_3	H
Petunidin	OCH_3	OH

Figure 2. The flavylium cation structure of anthocyanidins, which is named according to the B-ring substituents in C-3′ and C-5′.[24,63]

Anthocyanidins are, however, unstable as such. Therefore, in nature anthocyanidins occur almost exclusively, except for a few, as linked to a sugar moiety via an *O*-glycosidic bond of the hydroxy group of C-3 and a sugar (3-*O*-glycosides)[76] as glycosylation forms a stabilising intramolecular hydrogen bond network.[77] Examples of the non-glycosylated exceptions are 3-deoxyanthocyanidins not commonly found in foods.[64] The most common sugars in anthocyanins are glucose and rhamnose, but also other hexoses, such as galactose, other pentoses, such as xylose and arabinose, and disaccharides, such as rutinose and sambubiose, are found. The carbons C-5, C-7, C-3′ and C-5′ may carry an additional sugar moiety to form an anthocyanin diglucoside.[78] Also glycosylation of C-8 has been identified in certain flowers.[79] The anthocyanins of pigmented potatoes contain a glucose in C-5 (5-*O*-glucose) and in C-3, a rutinose (3-*O*-rutinose), which is a disaccharide containing a glucose and a rhamnose.[41,65,67,70,73]

The third building block of anthocyanins is the acyl group. The sugar moieties of acylated anthocyanins, usually in C-3 and C-5, may have a covalent ester linkage to one or more aliphatic or aromatic acids.[80] In pigmented potatoes, the rutinose in C-3 is linked to an aromatic hydroxycinnamic acid (*p*-coumaric acid, caffeic acid and ferulic acid).[46,41,65,67,70] An acylated anthocyanin commonly found in purple potatoes, petanin (petunidin 3-*O*-[6-*O*-(4-*O*-*E*-*p*-coumaroyl-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside]-5-*O*-*β*-D-glucopyranoside), is illustrated in the **Figure 3**.[41,65,67] The glucopyranoses are in a $^4C_1$ chair conformation, and the rhamnopyranoses are in $^1C_4$ chair conformation.[65] In addition, acylated residues have been found in potatoes, such as acylated diglucosides and derivatives without the glucose in C-5.[70] Some non-acylated anthocyanins, such as 3,5-diglucosides, have been detected.[70] However, these might be extraction degradants as the acyl linkages are more labile compared to

the glycosidic linkages leading inevitably to some degree of deacylation during the extraction process of acylated anthocyanins.[71,81,82]

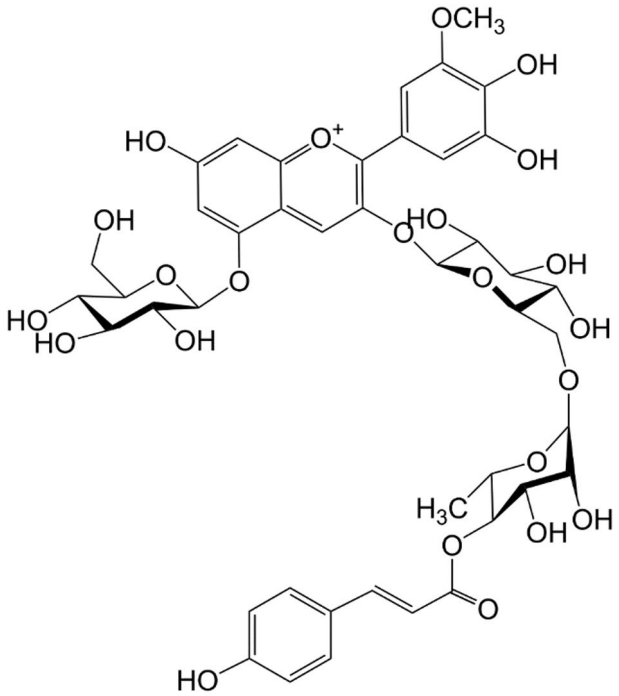


Figure 3. The structure of the common acylated anthocyanin found in purple potatoes, petanin. Adapted from Fossen et al., 2003.[67]

2.1.3 Accumulation and location of anthocyanins in tubers

Anthocyanins are formed in potato plants via the phenylpropanoid and flavonoid pathways described in detail elsewhere.[60,62] The biosynthesis occurs while transporting the anthocyanin precursors, such as tyrosine and phenylalanine, from the leaves to the tubers, where the final products, anthocyanins, accumulate.[83] The biosynthesis of anthocyanins has been reported to continue throughout the developmental process of potato tubers, but the colour intensity of the epiderm is reported to decrease.[59,84] In a tuber, anthocyanins are located in the vascular bundle or the parenchyma tissues (the cortex, the perimedullary zone, the central pith) (**Figure 4**).[54]

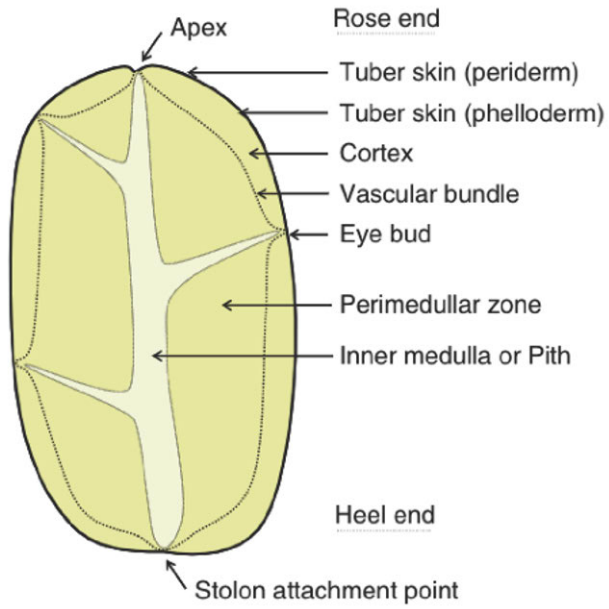


Figure 4. Morphology of a potato tuber. Reprinted from Potato Biology and Biotechnology, 1st edition, van Eck, H. J., Chapter 6: Genetics of morphological and tuber traits, p. 97, Copyright (2007), with permission from Elsevier.

2.1.4 Other edible plants containing acylated anthocyanins

In addition to pigmented potatoes, acylated anthocyanins with a similar anthocyanidin-3-hydroxycinnamoyl-rutinoside-5-glucoside structure are found in other edible anthocyanin-pigmented genera of the *Solanaceae* family. For example, pigmented peppers, tomatoes and aubergines contain similar anthocyanins but with delphinidin as the major anthocyanidin.[60,85–87] Other types of acylated anthocyanins may be found from several edible pigmented berries, fruits and vegetables, such as the purple sweet potato, red radish, purple carrots and red cabbage[23,88], briefly reviewed in **Table 1**. Examples of the acyl groups present in the acylated anthocyanins of edible foods are acetic, caffeic, coumaric, hydroxybenzoic, malonic, oxalic, sinapic and succinic acids, and the percentage of acylated anthocyanins as a proportion of the total anthocyanins vary, depending on the edible plant.[28,30,89–96] Overall, the acyl moieties and sugars and their number and location bring about great structural diversity.

Table 1. Examples of acylated anthocyanins and their structural diversity found in edible berries, fruits and vegetables. The proportion of the acylated anthocyanins as part of the total amount of anthocyanins is given as a percentage.

	Types of acylated anthocyanins	Acyl groups	Major acylated anthocyanins	Proportion (%)	Ref.
Berries					
Blueberry (lowbush)	Cy/dp/mv/pn/pt-3-acyl-glu	Ace, mal	NA	NA	[89]
Grape (*Vitis labrusca* 'Concord')	Cy/dp/mv/pn/pt-3-(6″-acyl-(di)glu)	Ace, cou	Dp-3-coum-5-diglu, pt-3-cou-5-diglu	38	[90]
Fruits					
Blood orange (*Citrus sinensis* L. cv. Sanguinelli)	Cy/dp/pn-3-(di)acyl-glu; cy-3-mal-dioxaloyl-glu	Oxa, mal	Cy-3-mal-glu	69	[91]
Passion fruit (*Passiflora suberosa*)	Cy/dp/pg/pt-3-6″-mal-glu	Mal	Pt/cy/dp-mal-glu	27	[92]
Vegetables and tubers					
Broccoli sprouts (*Brassica oleracea* 'Viola')	Cy-3-(di)acyl-diglu-5-glu, cy-3-diacyl-diglu-5-mal-glu	Cou, fer, mal, sin	Cy-3-sin-sin-diglu-5-glu; cy-3-sin-sin-diglu-5-mal-glu	NA	[93]
Pigmented potatoes (*Solanum tuberosum* L.)	Cy/dp/mv/pg/pn/pt-3-acyl-rut-5-glu	Caf, cou, fer	Pt-3-cou-rut-5-glu (purple potatoes), pg-3-cou-rut-5-glu (red potatoes)	98	[41,68,70]
Purple carrot (*Daucus carota*)	Cy-3-(2″-xyl-(6″-acyl-glu)-gal	Cou, fer, sin	NA	86	[30]
Purple corn (*Zea mays* L.)	Cy/pn/pg-3-(di)acylglu	Mal, suc	Cy-3-mal-glu	30	[94]
Purple sweet potato (*Ipomoea batatas* var. P40)	Cy/pn-3-(di)acyl-sop-5-glu	Caf, fer, Hba	Pn-3-caf-sop-5-glu, cy-3-caf-hba-sop-5-glu, cy-3-caf-fer-sop-5-glu	91	[95]
Red cabbage (*Brassica oleracea* L. var. capitata)	Cy-3-(di)acyl-di/triglu-5-glu	Caf, cou, fer, hba	Cy-3-sin-diglu-glu, cy-3-sin-sin-diglu-glu	79	[28]
Red lettuce (*Lactuca Sativa* L.)	Cy-acyl-glu	Mal	Cy-mal-glu	NA	[96]

Anthocyanidins: cy, cyanidin; dp, delphinidin; mv, malvidin; pg, pelargonidin; pn, peonidin; pt, petunidin. Acyl moieties: ace, acetyl; caf, caffeoyl; cou, coumaroyl; hba, hydroxybenzoyl; mal, malonyl; oxa, oxaloyl; sin, sinapoyl; suc, succinyl. Sugar moieties: glu, glucoside; gal, galactoside; rut, rutinoside; sop, sophoroside; xyl, xyloside. NA, not available.

2.2 Postprandial structural modifications of anthocyanins

2.2.1 Stability under physiological conditions

Anthocyanins as reactive compounds have compromised structural stability susceptible to various factors such as temperature, pH, light, copigmentation and ascorbic acid amongst others.[97–99] In the edible plants, anthocyanins are stable in the mildly acidic conditions of the vacuoles, but consuming anthocyanin-rich food, excluding here the effects of plant endogenous enzymes and the preparation of the food, exposes anthocyanins to varying pH conditions, physiological temperature and enzymes of both human and gut microbiota origin in the gastrointestinal tract. This leads to changes in the structures of anthocyanins. The reactivity of anthocyanins gives rise to their multifaceted postprandial metabolism and thus introduces severe technical and analytical challenges to postprandial investigations.

Anthocyanins undergo pH-dependent reactions (**Figure 5**). In the acidic (pH <2) aqueous media, anthocyanins occur predominantly in their stable structure: the red-coloured flavylium cation. When the pH rises, the flavylium cation undergoes two reactions. At pH 2–4, the flavylium cation is deprotonated to form a neutral blue quinoidal base. At pH 4–6, a nucleophilic attack by a water molecule (hydration) to the electrophilic C-2 of the flavylium cation followed by a proton loss takes place to form a tautomeric equilibrium between two neutral molecules, a colourless carbinol pseudobase and a yellow *cis*-chalcone. Additionally, *trans*-chalcones are formed from the *cis*-chalcones via isomerization forming equilibrium. Elevated temperatures enhance the stability of chalcones favouring their formation over flavylium cation, quinoidal base and carbinol pseudobase and thus promoting the loss of colour.[100–105]

In the human gastrointestinal tract, the saliva in the mouth introduces the first neutral physical environment (pH 6–7)[106] for anthocyanins during consumption. The pH of the stomach is around 2 in a fasting state, but rapidly increases even up to 5[107] or 7[108] after a meal. Dressman et al. suggested that the rise is possibly due to the buffering effect of ingested food.[108] The duodenum, the upper part of the small intestine, is neutral in the fasting state (pH 6) but the pH decreases slightly to 5 in the postprandial state.[108] The structure of anthocyanins in each part of the gastrointestinal tract is still unknown, but it has been suggested that the flavylium cation predominates only in the acidic stomach.[64] In the intestine and colon, the neutral unstable quinoidal base and chalcone structures may prevail instead of the flavylium cations.[109]

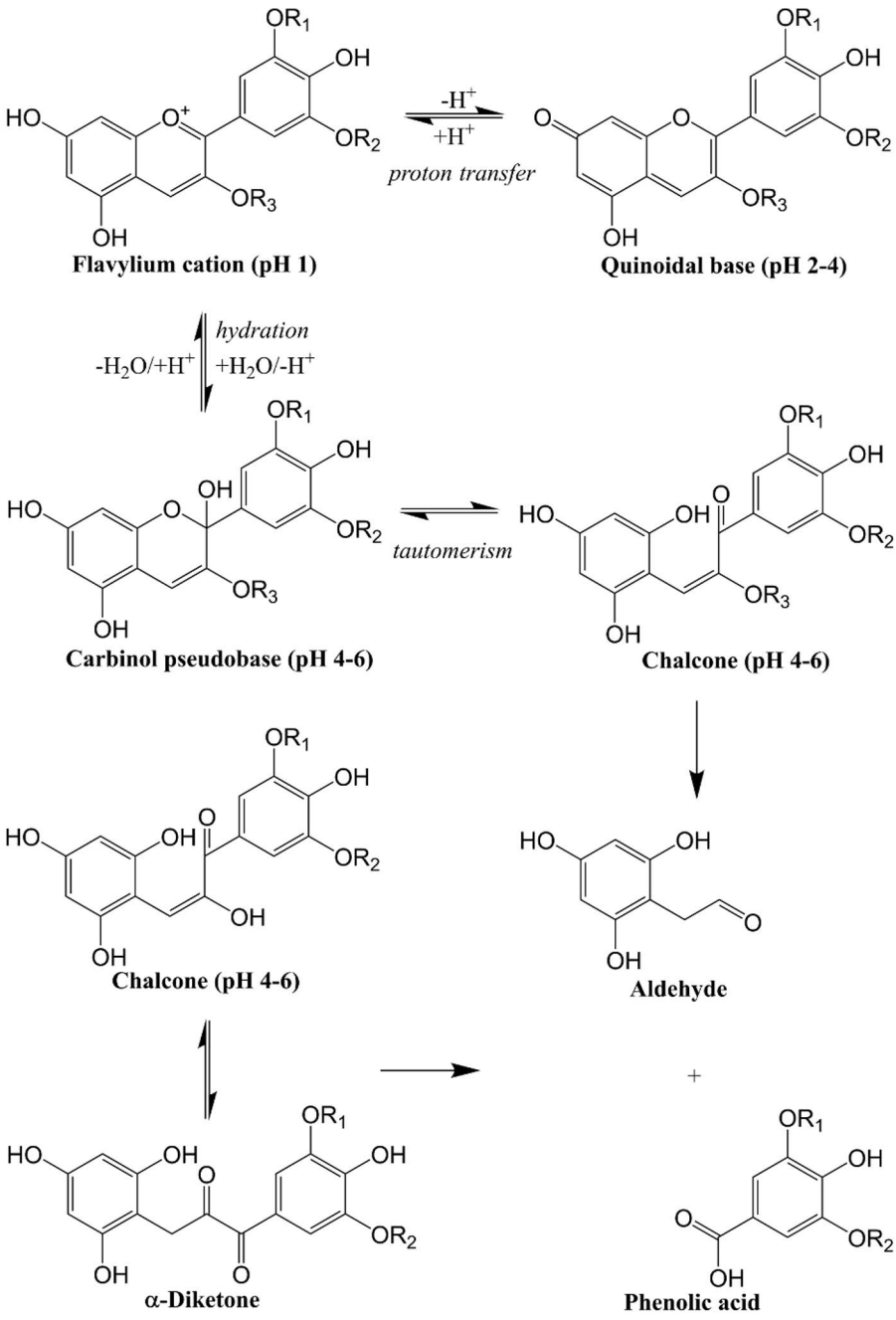


Figure 5. The pH-dependent chemical structures of anthocyanins. The R_1 and R_2 are the hydroxy- and methoxysubstituents of each anthocyanidin, and R_3 may be a glycosyl or a hydroxy group.[102,110]

Anthocyanidin aglycones are reported to degrade rapidly in physical conditions mimicking the human gastrointestinal tract *in vitro* (pH 7.4 and 6.4, 37 ºC) even without the presence of digestive and microflora enzymes.[31,109] In neutral conditions, chalcones are further degraded into stable breakdown products; a phloroglucinaldehyde and a phenolic acid whose substitution pattern corresponds to the anthocyanidin in question. The degradation of anthocyanidins occurs via equilibrium with an α-diketone, whereas glycosylation prevents the α-diketone from degradation (**Figure 5**). Instead of degradation, anthocyanidins may also dimerise via the quinoidal base under the physiological pH. Hydroxy- and methoxysubstituents in the B-ring decrease the stability of anthocyanidin aglycones and thus increase the degradation in neutral conditions. Glycosyl and acyl moieties, however, stabilise the anthocyanidins even under neutral pH conditions.[109]

Considering the monoacylated anthocyanins of pigmented potatoes, the pH-dependent structural changes of the monoacylated anthocyanins of purple and red potato extracts have been studied by Reyes et al. at room temperature. The aqueous extracts of potato anthocyanins are red until pH 3, colourless at 4–7 and pale yellow-green at 8–10, and in a one month storage period 68% and 86% of the colour intensity was retained for purple and red potatoes, respectively.[49] The monoacylated anthocyanin of purple potatoes, petanin (see Chapter 2.1.2, **Figure 3**) as compared to a common anthocyanin found in berries, cyanidin-3-*O*-glucoside, shows higher colour stability and intensity at pH 4–8.1 after 1 h in a buffer at 10 ºC and 23 ºC. The highest colour intensity appeared at pH 1.0 for cyanidin-3-*O*-glucoside and, surprisingly, at pH 8.1 (λ_{max} 577 nm) for petanin. Furthermore, petanin showed better stability during storage (pH 4, 10 ºC); for example, after 60 days, 84% was still intact, whereas the cyanidin-3-*O*-glucoside was totally degraded. The petanin was concluded to have similar stability when compared to the literature values for other anthocyanins acylated with an aromatic mono- or diacyl moiety.[111]

Acylated anthocyanins are generally considered to be more stable than the non-acylated ones due to their intramolecular and/or intermolecular copigmentation and self-association reactions.[21,23,111–114] Moreover, anthocyanins carrying two or more acyl groups are more stable than the monoacylated ones.[76] Polyacylated anthocyanins form intramolecular sandwich-type stacking due to hydrophobic interactions where two aromatic acids shelter the anthocyanidin nucleus from nucleophilic attack of water to C-2.[112,115] Monoacylated petanin, however, has not been detected to form intramolecular sandwich-type stacking of the *p*-coumaroyl and the anthocyanidin aglycone reported with polyacylated anthocyanins. Stability of petanin is achieved by the intermolecular stacking of petunidin aglycones as well as *p*-coumaroyl moieties to prevent the hydration of C-2 and, thus, the formation of the colourless

chalcones. Petanin has been suggested to form a dimer.[114,116] However, the extent of inter- and intramolecular copigmentation and self-association of acylated anthocyanins under physiological conditions have not been studied.

2.2.2 Absorption, metabolism and excretion

In addition to the structural modifications driven by the physiological environment, anthocyanins are also subjected to digestive enzymes and xenobiotic phase I and II metabolism. Phase I reactions include, for example, oxidation and reduction, whereas phase II metabolism involves conjugation reactions with methyl, glucuronic acid and sulfate groups in the enterocytes of small intestine and hepatocytes of liver.[117] The absorption, biotransformation and excretion of anthocyanins are discussed next with a special interest in the acylated structures whenever possible.

In *the mouth*, chewing anthocyanin-rich food with the teeth releases the anthocyanins from the vacuolar structures and mixes the food bulk with saliva. In *ex vivo* studies of the structural modifications of anthocyanins in human saliva, anthocyanins have been hydrolysed into their aglycones.[118–120] Nevertheless the spontaneous reactions related to pH and physiological temperature described in the previous chapter, the primary hydrolysis mechanism is enzymatic as the heat-inactivated saliva, cell-free saliva and enzyme-free artificial saliva showed less degradation of cyanidin glycosides compared to intact saliva at a physiological pH and temperature.[119] The β-glucosidase of saliva, oral epithelium and oral microbiota are responsible for the hydrolysis reactions[118] of which microbiota contributes the most at 37 ºC both *in vivo* and *ex vivo*.[119,120] The sugar moiety and the anthocyanidin aglycone affect the efficiency of the hydrolysis reaction; incubation of anthocyanin glycosides in human saliva *ex vivo* degraded more glycosides of delphinidin and petunidin than those of cyanidin, malvidin and peonidin.[119] In the oral cavity of humans, the loss of cyanidin xyloside from chokeberry juice was greater when compared to galactoside, glucoside and arabinoside of cyanidin, and delphinidin glucoside in red grape juice, which degraded more than the glucosides of cyanidin, petunidin, peonidin and malvidin.[120] Monosaccharides are more susceptible to hydrolysis in saliva when compared to di- and trisaccharides as detected after *ex vivo* incubation in human saliva.[119] In another *ex vivo* study conducted with human saliva, mono- and diacylation stabilized the incubated purple sweet potato anthocyanins from degradation.[121]

The stomach is an important site of absorption for anthocyanin glycosides. This was evidenced by several authors detecting anthocyanins in plasma shortly after ingestion, both in human volunteers[122–124] and animal models[125–129]. The molecular mechanism behind the transport of anthocyanins from the lumen of

the stomach into the gastric mucosa involves an organic anion membrane carrier, bilitranslocase. It is located in the epithelial cells of the gastric mucosa, and its substrates are mono- and diglycosylated anthocyanins. However, deglycosylation or acylation of monoglycosylated anthocyanins were reported to decrease the transporting activity *in vitro*.[130] *In vitro* studies with a gastric cell model, MKN-28 cell line, show that the glucose transporters GLUT1, GLUT3 and monocarboxylated transporter MCT1 may contribute to the gastric uptake of anthocyanins by binding the B-ring or glucose as studied with red wine anthocyanins.[131] However, multiple glycosyl and acyl groups hinder the transportation, as detected with purified mono- and diacylated purple sweet potato anthocyanins compared to an anthocyanidin glucoside, malvidin-3-*O*-glucoside.[132] Furthermore, in simulated gastric conditions *in vitro*, the acylated anthocyanins of purple potatoes[133], purple sweet potatoes[121,134] and red cabbage[135] are stable and are further released from the food matrix[135].

The gastric absorption of petanin has not been investigated, but an acylated anthocyanin with a similar structure, nasunin from aubergine (delphinidin-3-*p*-coumaroyl-rutinoside-5-glucoside), peaked rapidly as intact in rat plasma 15 minutes after oral administration, showing similar absorption with delphinidin-3-glucoside.[136] A malvidin coumaroyl diglucoside extracted from red wine showed similar absorption in a human gastric cell model, MKN-28, as its non-acylated counterpart *in vitro*.[137] Likewise, a cyanidin-derived monoglycoside acylated with a malonic acid was similarly absorbed as cyanidin-3-glucoside in rats.[138] Furthermore, the major anthocyanin of the purple sweet potato, peonidin-3-caffeoyl-sophoroside-5-glucoside, was absorbed as such in rats.[139] After ingesting purple sweet potato extract containing eight acylated anthocyanins, only two of them, cyanidin and peonidin derivatives of caffeoyl-sophoroside-glucoside, were rapidly absorbed in rats peaking at five minutes after ingestion and thus indicating the stomach as the site of absorption.[140] Moreover, the same two anthocyanins were seen in human plasma, but the six other ingested diacylated anthocyanins were not, suggesting structure-dependent absorption.[140]

The small intestine introduces a similarly important absorption site for anthocyanin glycosides such as cyanidin glucoside and rutinoside, as detected in rat studies.[141,142] *In vitro*, a mouse jejunum set into a Ussing chamber has been shown to be the major absorptive part of the small intestine for cyanidin glucoside with only a little contribution from the duodenum and none from the ileum.[143] Anthocyanin glycosides are absorbed *in vitro* into an everted rat jejunum sac model as detected with cyanidin glucoside and cyanidin rutinoside, but the corresponding aglycone, cyanidin, is not. Passive diffusion of the aglycone did not occur regardless of its high lipophilicity.[142] The transport mechanism of the anthocyanin glycosides to the enterocytes may involve the brush-border glucose transporters GLUT2 and SGLT1 (sodium-glucose

cotransporter).[142,144–146] *In vivo*, Mülleder et al. showed evidence of the intestinal glucose transportation system in healthy volunteers as a simultaneous consumption of glucose and elderberry extract rich in cyanidin-3-glucoside and cyanidin-3-rutinoside resulted in a lower excretion of anthocyanins into the urine when compared to the extract alone.[146] Glucose transporters are also involved in the transport of acylated anthocyanins, as glucose inhibits the absorption of acylated purple sweet potato anthocyanins *in vitro.*[121] The acylated purple potato and purple carrot anthocyanins are absorbed (6±1% and 36±6%, respectively) into human intestine cell model, Caco-2, via glucose transporters.[147]

The structures of anthocyanins affect their transport efficiency; more methoxygroups and less hydroxygroups in the B-ring enhances the transport of anthocyanins to Caco-2 cells *in vitro* as the transportation of malvidin glucoside was the best and that of delphinidin glucoside was the least efficient among the seven blueberry anthocyanins studied (glucosides of cyanidin, delphinidin, petunidin, peonidin and malvidin, galactosides of cyanidin and peonidin). The glucosides were absorbed more than the galactosides.[148] *In situ* perfusion in rats, however, showed that cyanidin glucoside was absorbed more than malvidin glucoside, and cyanidin rutinose was absorbed less than cyanidin glucoside.[141] Acylation (a malonic acid) of cyanidin glucoside interestingly favoured the intestinal absorption over non-acylated cyanidin glucoside in rats.[138] *In vitro*, malvidin-coumaroyl-diglucoside was absorbed similarly as its non-acylated counterpart in human enterocyte Caco-2 model cells.[137] In healthy volunteers, acylated anthocyanins from purple carrots were shown to have a shorter mathematically modelled half-life of absorption to plasma in the upper gastrointestinal tract than the non-acylated ones, suggesting a narrower absorption site of acylated anthocyanins.[149] Furthermore, the lactase phloridzin hydrolase (a β-glucosidase), may hydrolyse anthocyanin monoglucosides in the small intestine as Hassimotto et al. detected hydrolysis of cyanidin glucoside, but not cyanidin rutinoside, in rats.[142] Acylated anthocyanins of purple potato, purple sweet potato and red cabbage may be hydrolysed and/or spontaneously degraded as their amount decreased in a vessel simulating small intestine without a cellular absorption model.[133–135]

The colon is not known to absorb anthocyanins.[143] However, the colon is an essential part of the postprandial metabolism of anthocyanins as large amounts of non-absorbed anthocyanins reach the colon and are exposed to microbial and endogenous β-glycosidase.[31,142] Intact acylated anthocyanins from the purple potato and purple carrot may reach the colon (71.8±0.3% and 45±1%, respectively) as studied in an *in vitro* gastrointestinal model.[147] The colonic microbiota has been detected to rapidly hydrolyse anthocyanin glycosides, such as glucosides, diglucosides and rutinosides, as investigated *in vitro* by fermenting the isolated gut microbiota of pig caecum[31] and faecal suspensions of

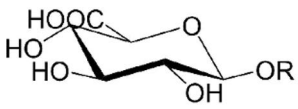
rats[142] and human volunteers[109,150–152] with anthocyanins of interest. Microbiota also breaks down the heterocyclic C-ring and produces small phenolic compounds.[150,152] Furthermore, as the hydrolysed aglycones are not stable in the neutral pH of the colon, they are broken down to phenolic acids corresponding to the substitution pattern of the B-ring of the parent anthocyanidin in question; for example, protocatechuic acid is formed from cyanidin, vanillic acid from peonidin and syringic acid from malvidin.[31,109,142] Other phenolic metabolites resulting from the microbial activity include *e.g.* catechol, pyrogallol, hydroxybenzoic acids, propionic acids, gallic acid and *p*-coumaric acid.[151,152] The phenolic metabolites show enhanced stability under neutral pH conditions as compared to anthocyanins, and methoxysubstituted phenolic acids may be *O*-demethylated, thus possibly leading to increased antioxidant properties.[31] The uptake mechanism of these metabolites (*e.g.* benzoic and hydroxybenzoic acid) may involve MCT1.[153]

The acylated anthocyanins may be more readily degraded by the gut microbiota than the non-acylated. After a 12-week chronic intake of a Concord grape supplement rich in acylated anthocyanins by obese mice, the content of total anthocyanins in faeces was increased by a tenfold compared with the faeces collected during a late test week when antibiotics were administered to knock down the gut microbiota. The change was greater than that observed after the intake of berry supplements rich in non-acylated anthocyanins.[154] Cyanidin glucoside acylated with a malonic acid is likely to be more readily available for microbial degradation in rats than its non-acylated counterpart as the ratio of these two anthocyanins in the caecal contents was changed to favour the non-acylated anthocyanin when compared to the ratio in the food origin, red orange juice.[138] Human gut microbiota is able to break down acylated anthocyanins as seen by Fleschhut et al. for pelargonidin sophorosides acylated with hydroxycinnamoyl and malonyl moieties, leading to 4-hydroxybenzoic acid and hydroxycinnamic acids *in vitro*.[109] The acylated anthocyanins of purple potatoes and red cabbage were degraded by the human gut microbiota in an *in vitro* gastrointestinal model.[133,135] *In vitro* incubation of Concord grape juice rich in both non-acylated and acylated anthocyanins (*O*-acetyl glycosides, *O*-*p*-coumaroyl-*O*-diglucosides, *O*-*p*-coumaroylglucosides) in human faeces resulted in a variety of phenolic acids, such as phenylacetic acids, phenyllactic acids and hydroxybenzoic acids, and simple phenolic degradants catechol, resorcinol, pyrogallol and phloroglucinol.[155]

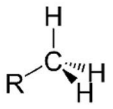
Phase I and II metabolism reactions occur in addition to deglycosylation. In a human clinical intervention, pelargonidin was detected in human urine even though this anthocyanidin was not ingested, suggesting the interconversion of anthocyanidins during postprandial metabolism.[156] This may be due to cytochrome P450 isoforms which remove methyl groups and add hydroxy

groups (phase I), and in phase II metabolism, methyl groups may be added to hydroxy groups to form methoxygroups.[156,157] The phase II mechanism is an important pathway occurring in the intestinal epithelial cells of small intestine and colon walls.[25,157] The anthocyanin glucuronides may be transferred through the gastric and small intestine walls, possibly with the same transportation mechanisms similar to the non-conjugated anthocyanins, as malvidin glucuronide appeared in the basolateral side of *in vitro* model cells with similar transport efficiency as non-conjugated malvidin (di)glucosides.[137] Anthocyanins are conjugated to a large extent with a glucuronic acid via uridine 5′ diphosphate-glucuronosyltransferases, a methyl group via catechol-*O*-methyl transferase, and a sulfate via sulfotransferase[39], as detected in several studies involving human volunteers (**Figure 6**).[126,156,158,159] The urinary concentration of the anthocyanin degradants and conjugates exceeds the amount of parent compounds; a 24-fold increase was detected after an intake of bilberry juice by healthy volunteers.[156]

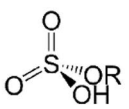
Acylated anthocyanins may also undergo methylation in phase II metabolism, as cyanidin-malonyl-glucoside was methylated in rat metabolism and excreted via urine like its non-acylated counterpart[138], and methylated monoacylated (caffeic, sinapic and ferulic acids) cyanidin triglucosides were found in postprandial plasma and urine of healthy volunteers after a meal of red cabbage[160]. Glucuronidation of intact acylated anthocyanins has not been reported; for example nasunin was not methylated nor glucuronidated in rat metabolism.[136] Clinical interventions feeding red cabbage and grape juice rich in both acylated and non-acylated anthocyanins report only non-acylated anthocyanins conjugated with a methyl, glucuronide or diglucuronide, giving evidence that the acyl groups may be cleaved prior to conjugation and only the non-acylated anthocyanins undergo conjugation.[28,90,160] The phenolic degradants are conjugated in phase II metabolism as well, as detected with *in vitro* gastrointestinal model[161] and in human volunteers.[159]



Glucuronyl group



Methyl group



Sulfonyl group

Figure 6. The phase II conjugate groups of anthocyanins (R).

Enterohepatic circulation has been suggested to occur due to the persistence of anthocyanin conjugates in human urine up to 24 hours[156,158,162] and 48 hours[159], and the second rise of anthocyanin concentration in plasma.[25] Generally, the absorbed xenobiotics in the portal vein are transferred to the liver, in which phase II conjugation groups are attached. Then, they are transported back to the small

intestine via bile instead of releasing to systemic circulation. In the small intestine, the metabolites may be deconjugated, absorbed and transported to the liver.[163] In animal studies, anthocyanins and anthocyanin phase II conjugates have been detected from the liver after absorption[125,129,164–166], as transferred from the portal circulation via bilitranslocase[164], and from bile[165]. Liver may continue the phase II conjugation of anthocyanins, as detected in a rat study reporting methylation of the B-ring of cyanidin-3-glucoside (catechol) in liver, possibly with catechol-*O*-methyltransferase.[125] Liver may also break down anthocyanins to phenolic acids and further produce their phase II conjugates as studied with human liver microsomes.[161] From enterohepatic circulation, anthocyanins and their metabolites are transferred to systemic circulation.

In *blood (plasma or serum)*, anthocyanins appear quickly; the maximum concentration (C_{max}) of anthocyanins in humans is reached within 0.50–4 hours after an anthocyanin-rich meal in nanomolar concentrations (**Table 2**). In humans, anthocyanins have been found as such and, for example, also as glucuronides of both aglycones and glycosides, sulfated aglycones and methylated aglycone and glycoside glucuronides.[40,159,160,167–171] Some acylated anthocyanins of purple sweet potatoes (two out of eight)[140] and red cabbage (16 out of 18)[160] have been detected in clinical trials. Systemic circulation transports anthocyanins further to target tissues such as the kidneys[125,129,165,166], brain[129], lung, heart and prostate[165] as studied with rodent models. The phenolic metabolites detected in plasma are discussed in the following chapter.

Urine, faeces and breath are the excretion routes of anthocyanins and anthocyanin metabolites in humans as detected after acute consumption of $^{13}C_5$-cyanidin-3-*O*-glucoside.[158,159] The major excretion route at 0–6 h was urine, whereas at 6–24 h it was faeces. The maximum rate of elimination in breath occurred at 6 h. Furthermore, the urinary concentrations of parent anthocyanins exceeded those in plasma by 30-fold.[159] The urinary recoveries of the parent anthocyanins in healthy humans after one meal are well below 1%, which is further decreased by acylation, and the t_{max} varies from 0.75–5 hours (**Table 2**). In urine, anthocyanins have been detected both as aglycones and glycosides and their glucuronides and methyl glucuronides, and also as aglycone diglucuronides and anthocyanin (di)sulfates, but mostly as aglycones with glucuronidation or methylation.[40,126,156,167,169,170,172–174] Clinical trials feeding purple potatoes[175] and Concord grape juice (38% of acylated anthocyanins)[90] did not detect acylated anthocyanins in human urine, but other trials detected acylated anthocyanins of the purple sweet potato (two out of eight[140] and one out of seven[176]), red cabbage (eight out of 30[28] and 16 out of 18[160]) and purple carrot (two out of three)[30] have been detected, suggesting structure-dependent absorption. In rats, a 3-month feeding with grape containing a mix of acylated (22%) and non-acylated

anthocyanins lead to detection of one acylated anthocyanin out of three (petunidin-3-coumaroyl-glucoside).[177]

Table 2. A short review of the pharmacokinetics of anthocyanins after one meal in healthy volunteers. The recoveries of acylated anthocyanins are **bolded**.

Food	*n*	Dose (mg)	Recovery (%, urine)	t_{max} (h, urine)	C_{max} (nM, plasma)	t_{max} (h, plasma)	Ref.
Bilberry (extract)	5	2 225	0.03 / 24 h	2–4	27±11a 43±21b	1.4±0.5 2.2±1.1	[34]
Black currant (juice)	14	716	0.048 / 4 h	NA	NA	0.75	[178]
		1239	0.07 / 4 h	NA	NA	0.75	
		746e	0.045 / 4 h	NA	NA	1.5	
Blueberry (powder)*	5	1200f	0.002–0.003 / 4 h (plasma)	NA	29.1±6.1	4	[179]
Elderberry (capsule)	26	500	NA NA	2–3d 2–3e	7c 16d	2d 2e	[40]
Purple carrot (juice)*	10	65	NA	NA	2.5±0.6	2	
		194	NA	NA	6.6±1.3	1	
		323	NA	NA	9.6±1.7	2	
Purple carrot (raw or cooked)*	12	208 (raw)	0.140, **0.013** / 24 h	4	5.8±1.7	2	[30]
		160	0.190, **0.014** / 24 h	4	5.3±1.9	2	
		320	0.100, **0.008** / 24 h	4	5.0±1.4	2	
Red grape (juice)*	9	284	0.23	0.5	223	0.5	[180]
Red wine	9	280	0.18	1.5	96	1.5	[180]
Purple sweet potato (beverage)*	6	311	**0.01–0.03** / 24 h	0–5	2.0	1.5	[140]
Red cabbage (microwave-cooked)*	12	62	0.176, **0.041** / 24 h	2–4	NA	NA	[28]
		124	0.105, **0.023** / 24 h	2–4			
		186	0.085, **0.020** / 24 h	2–4			

C_{max}, maximum concentration; t_{max}, time point of maximum concentration; ref., reference; *contains acylated anthocyanins; NA, not analysed; a, malvidin-3-*O*-glucoside; b, peonidin-3-*O*-glucoside; c, cyanidin-3-*O*-glucoside; d, cyanidin-3-*O*-sambubioside; e, with carbohydrates; f, with high-fat meal.

Absorption of anthocyanins in humans may be affected by several factors, such as structural characteristics of the anthocyanins[149,178,181], dosage[28–30,174], simultaneously ingested other foods and nutrients[170,178], food matrix and food processing[29,30,32,160], as reviewed elsewhere[182]. For example, the t_{max} of non-acylated anthocyanins was delayed due to the simultaneous ingestion of a rice cake or cream when black currant juice[178] or strawberries[170], respectively, were consumed by healthy human volunteers. The t_{max} of urinary phenolic metabolites was delayed when a berry purée rich in non-acylated anthocyanins was consumed with cereals when compared to the consumption of berry purée alone.[32] Furthermore, the bioavailability of anthocyanins of fresh red cabbage was 10% higher than that of fermented cabbage.[160] The food matrix did not affect the recovery of acylated cyanidin-based anthocyanins of purple carrots whether

the carrots were served raw, microwave-cooked or juiced.[29,30] The non-acylated counterparts, however, were recovered less from plasma and urine after microwave cooking as compared to raw carrots.[30] Even though the whole carrots and carrot juice provided similar amounts of total anthocyanins in the plasma, the anthocyanins were absorbed quicker after the meal of carrot juice.[29,30]

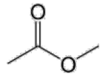
2.2.3 Phenolic metabolites

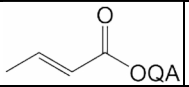
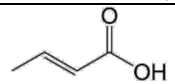
Recent acute clinical trials have shown evidence that *in vivo*, anthocyanins are degraded into phenolic metabolites spontaneously and/or enzymatically. These phenolic metabolites of anthocyanins consist of a variety of phenolic acids, aldehydes, alcohols, acetic acids, propanoic acids and hydroxycinnamic acids, which may be further subjected to phase II conjugation.[34,40,158,159] The total concentration of these metabolites is high; a 42-fold[159] and 45-fold[40] (plasma) and 60-fold[40] (urine) increase has been reported when compared to the parent anthocyanins after an intake of elderberries[40] or ^{13}C-labelled cyanidin-3-*O*-glucoside[159].

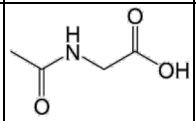
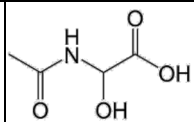
Clinical investigations of the phenolic metabolites of acylated anthocyanins are limited. Until the studies described in the experimental part of this thesis, only one study had analysed some of the urinary phenolic metabolites of five healthy volunteers after a meal of purple potatoes rich in acylated anthocyanins but reported uncertain identifications.[175] Two studies had investigated the phenolic metabolites of Concord grape juice containing a mix of non-acylated and acylated anthocyanins.[90,155] Due to this gap in the knowledge, the phenolic metabolites of foods containing mainly non-acylated anthocyanins are summarised in **Table 3** from the relevant postprandial clinical interventions (2009–2020) in which healthy volunteers consumed a meal rich in anthocyanins. However, drawing conclusions about the degradation of structurally different anthocyanins into phenolic metabolites on the basis of the current literature is challenging as the reviewed studies differ in study design and analytical methods. For example, they vary in the anthocyanin source and food matrix (purified anthocyanin, anthocyanin-rich extract or whole food), dose of anthocyanins, type of anthocyanins (one or mixed), type of meal (anthocyanin source alone or a mixed meal) and other compounds in the dietary anthocyanin source possibly metabolising similarly and/or affecting the absorption and metabolism of anthocyanins.

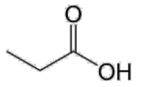
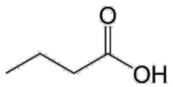
Table 3. Suggested phenolic metabolites of anthocyanins and anthocyanin-rich foods reported in recent clinical interventions (2009–2020) serving an acute anthocyanin-rich meal to healthy volunteers. The metabolites, consisting of a substituted benzene ring, were identified using HPLC-MS, apart from one study[90] (**m**) utilising GC-MS. The reference studies were conducted with berry meals rich in non-acylated anthocyanins except for studies **a**[158] and **e**[159] (^{13}C-traced cyanidin-3-*O*-glucoside), study **j**[175] (purple potatoes) and studies **l**[90] and **m**[155] (grape juice containing both non-acylated and acylated anthocyanins). Studies **a**[158], **b**[183] and **e**[159] contain investigation of faecal metabolites. Abbreviation s means a sulfate and glc a glucuronide (see **Fig. 6** for the representative structures). The C_{max} and t_{max} refer to plasma samples.

	Benzene ring substituents			Functional group			Detected in:		
Metabolite	OH	OCH_3	Others	in C1 of benzene	C_{max} (nM)	t_{max} (h)	Urine	Blood	Faeces
Benzoic acids and hydroxybenzoic acids									
Benzoic acid	-	-	-		2169±608d	8.6±3.0d	d	d	-
2-Hydroxybenzoic acid	2-OH	-	-		308±128d	6.3±3.3d	bd	bd	-
3-Hydroxybenzoic acid	3-OH	-	-		66±23d	6.1±2.4d	abdm	bd	a
3-Hydroxybenzoic acid glucuronide	-	-	3-*O*-glc		-	-	g	-	-
3-Hydroxybenzoic acid sulfate	-	-	3-*O*-s		-	-	k	-	-
4-Hydroxybenzoic acid	4-OH	-	-		42±7d	5.3±2.5d	abcdfgk	bdg	ab
4-Hydroxybenzoic acid glucuronide	-	-	4-*O*-glc		-	-	g	-	-
4-Hydroxybenzoic acid sulfate	-	-	4-*O*-s		-	-	gk	-	-
2-Hydroxy-4-methoxybenzoic acid	2-OH	4-OCH_3	-		-	-	e	bce	be
2,3-Dihydroxybenzoic acid	2,3-OH	-	-		12024±4055d	7.3±3.2d	bdg	dg	ab
2,4-Dihydroxybenzoic acid	2,4-OH	-	-		22±4	5.1±2.4d	dg	d	-
2,5-Dihydroxybenzoic acid	2,5-OH	-	-		425±137	4.4±0.3d	d	d	-
2,6-Dihydroxybenzoic acid	2,6-OH	-	-		-	-	g	g	-
3,4-Dihydroxybenzoic acid (protocatechuic acid)	3,4-OH	-	-		146±74a; 109±45d; 81±32h; 7.6±1.8n; 4.5±0.5o;	3.3±0.7a; 2.0±0.6d; 1.0±0.0h; 57±5n; 63±8o;	ac–gik	a–e,g–i	abe

Metabolite	Benzene ring substituents			Functional group in C1 of benzene	C_{max} (nM)	t_{max} (h)	Detected in:		
	OH	OCH_3	Others				Urine	Blood	Faeces
Protocatechuic acid-3-*O*-glucuronide	4-OH	-	3-*O*-glc		11±3a	2.7±1.0a	abce	abce	abe
Protocatechuic acid-4-*O*-glucuronide	3-OH	-	4-*O*-glc		68±61a	3.8±0.8a	abce	abce	abe
Protocatechuic acid-3-*O*-sulfate	4-OH	-	3-*O*-s		157±116a*	11.4±3.8a*	abcge	abce	abe
Protocatechuic acid-4-*O*-sulfate	3-OH	-	4-*O*-s		157±116a*	11.4a±3.8*	abcge	abce	abe
3,5-Dihydroxybenzoic acid	3,5-OH	-	-		-	-	bc	bc	-
Gallic acid	3,4,5-OH	-	-		25±12h	1.4±0.5h	g	gh	-
3-*O*-Methyl gallate	3,4-OH	5-OCH_3	-		79±25h	1.0±0.0h	-	bh	-
4-*O*-Methylgallic-3-*O*-sulfate	5-OH	4-OCH_3	3-*O*-s		275±82d	2.1±0.3d	d	d	-
Syringic acid	4-OH	3,5-OCH_3	-		8±6d; 777±182h	0.3±0.2d; 1.0±0.0h	df	bcdh	-
Vanillic acid	4-OH	3-OCH_3	-		1845±838a; 410±115d; 379±111h	12.5±11.5a; 1.8±0.8d; 1.2±0.5h	b–gkm	a–dhe	abe
Vanillic acid-4-*O*-glucuronide	-	3-OCH_3	4-*O*-glc		24±4a	4.8±0.4a	abceg	abceg	abe
Vanillic acid-4-*O*-sulfate	-	3-OCH_3	4-*O*-s		430±299a*; 1054±274d	30.1±11.4a*; 2.1±0.8d	a–eg	a–de	abe
Isovanillic acid	3-OH	4-OCH_3	-		195a**; 220±44d	2.0a**; 9.8±3.6d	adeg	ade	abe
Isovanillic acid-3-*O*-glucuronide	-	4-OCH_3	3-*O*-glc		35±5a	4.3±0.6a	abceg	abceg	abe
Isovanillic acid-3-*O*-sulfate	-	4-OCH_3	3-*O*-s		-	-	abeg	be	abe
Benzoic acid methyl esters									
Methyl-3,4-dihydroxybenzoate	3,4-OH	-	-		12±5a	8.4±5.7a	aeg	abceg	abe
Methyl-4-hydroxybenzoate	4-OH	-	-		-	-	g	g	-
Methyl vanillate	4-OH	3-OCH_3	-		-	-	e	e	abe
Catechol derivatives									
Catechol-*O*-sulfate	1-OH	-	2-*O*-s		24555±3775d	7.1±0.4d	d	d	-

Metabolite	Benzene ring substituents			Functional group in C1 of benzene	C_{max} (nM)	t_{max} (h)	Detected in:		
	OH	OCH_3	Others				Urine	Blood	Faeces
4-Methylcatechol-*O*-sulfate	1-OH	-	2-*O*-s, 4-CH_3		3497±1192d	7.7±3.2d	d	d	-
Chlorogenic acid derivatives									
Chlorogenic acid	3,4-OH	-	-		5±2d	0.7±0.3d	d	d	-
Cinnamic acids and hydroxycinnamic acids									
Cinnamic acid	-	-	-		123±34d	6.2±2.4d	-	d	-
Caffeic acid	3,4-OH	-	-		1±1d	0.1±0.1d	dfg	dl	ab
Caffeic acid-3-*O*-glucuronide	4-OH	-	3-*O*-glc		16±4d	1.1±0.2d	d	d	-
Caffeic acid-4-*O*-glucuronide	3-OH	-	4-*O*-glc		59±8d	1.2±0.1d	d	d	-
Caffeic acid-3-*O*-sulfate	4-OH	-	3-*O*-s		-	-	kl	l	-
Caffeic acid-4-*O*-sulfate	3-OH	-	4-*O*-s		-	-	l	-	-
o-Coumaric acid	2-OH	-	-		6±1d	1.2±0.3d	d	d	-
m-Coumaric acid	3-OH	-	-		29±14d	3.2±0.8d	d	d	-
p-Coumaric acid	4-OH	-	-		131±51d	1.0±0.0d	bcdfgl	bdgl	b
p-Coumaric acid-4-*O*-glucuronide	-	-	4-*O*-glc		-	-	g	g	-
p-Coumaric acid-4-*O*-sulfate	-	-	4-*O*-s		-	-	g	-	-
Coumaric acid-*O*-sulfate	OH	-	*O*-s		-	-	l	-	-
Ferulic acid	4-OH	3-OCH_3	-		827±371a; 47±12d	8.2±4.1a; 1.0±0.0d	abcefgik	a–egil	abe
Ferulic acid-4-*O*-glucuronide	-	3-OCH_3	4-*O*-glc		165±29d; 18±2k	1.3±0.2d; 1.5k**	dgk	dgk	-
Ferulic acid-4-*O*-sulfate	-	3-OCH_3	4-*O*-s		2268±794d; 47±14k	3.6±2.6d; 1k**	dgkl	dkl	-
Isoferulic acid	3-OH	4-OCH_3	-		4592±2251d	3.8±2.5d	df	d	-
Isoferulic acid-3-*O*-glucuronide	-	4-OCH_3	3-*O*-glc		387±95d; 14±2k	1.9±0.6d; 1.5k**	dkl	dk	-
Isoferulic acid-3-*O*-sulfate	-	4-OCH_3	3-*O*-s		49±6d	4.1±2.5d	dkl	d	-

Metabolite	Benzene ring substituents			Functional group in C1 of benzene	C_{max} (nM)	t_{max} (h)	Detected in:		
	OH	OCH_3	Others				Urine	Blood	Faeces
Sinapic acid	4-OH	3,5- OCH_3	-		46±11d	1.9±0.8d	bcd	d	-
Hippuric acid derivatives									
Hippuric acid	-	-	-		1962±1389a; 42926±12282d	15.7±4.1a; 21.8±2.2d	abdgijk	abdgi	ab
2-Hydroxyhippuric acid	2-OH	-	-		7±2d	6.4±3.3d	d	d	-
3-Hydroxyhippuric acid	3-OH	-	-		45±7d	8.3±3.9d	dm	d	-
4-Hydroxyhippuric acid	4-OH	-	-		592±157d; 78±12k	3.8±3.8d; 1k**	dkm	dk	-
3-Methylhippuric acid	-	-	3-CH_3		-	-	b	-	-
4-Methylhippuric acid	-	-	4-CH_3		-	-	b	-	-
α-Hydroxyhippuric acids									
α-Hydroxyhippuric acid	-	-	-		2943±587d	3.9±2.5d	d	d	-
Phenolic alcohols									
4-Hydroxybenzyl alcohol	4-OH	-	-		-	-	bc	-	-
Phenolic aldehydes									
4-Hydroxybenzaldehyde	4-OH	-	-		667±653a; 77±18d	5.6±3.1a; 4.9±2.5d	adg	a–dg	ab
3,4-Dihydroxybenzaldehyde	3,4-OH	-	-		34±10d	4.1±2.6d	adg	bcdg	ab
4-Methoxybenzaldehyde	-	4-OCH_3	-		-	-	-	-	a
Phloroglucinaldehyde	2,4,6-OH		-		582±536a; 29±8h	2.8±1.1a; 2.4±0.9h	abc	bcgh	ab

Metabolite	Benzene ring substituents			Functional group in C1 of benzene	C_{max} (nM)	t_{max} (h)	Detected in:		
	OH	OCH_3	Others				Urine	Blood	Faeces
Phenylacetic acid derivatives									
Homoprotocatechuic acid	3,4-OH	-	-		476±138d; 180±89k	7.3±2.3d; 6k**	a–gk	dgk	abe
Homovanillic acid	4-OH	3-OCH_3	-		511±165d	5.6±0.9d	bcdfgkm	bdg	b
Homovanillic acid-4-*O*-sulfate	-	3-OCH_3	4-*O*-s		30±10d	10.2±3.5d	d	d	-
Homoisovanillic acid	3-OH	4-OCH_3	-		-	-	g	g	-
2-Hydroxyphenyl acetic acid	2-OH	-	-		-	-	g	g	-
3-Hydroxyphenyl acetic acid	3-OH	-	-		615±360d	10.9±3.4d	dfg	dg	-
4-Hydroxyphenyl acetic acid	4-OH	-	-		1849±724d	13.8±4.0d	adeg	dg	ae
Phenylacetic acid	-	-	-		8304±1886d	9.4d	d	d	-
Phenylpropanoic acid derivatives									
Dihydrocaffeic acid	3,4-OH	-	-		93±32d	7.6±3.3d	dflm	-	-
Dihydrocaffeic acid-3-*O*-glucuronide	4-OH	-	3-*O*-glc		84±11d	22.2±1.8d	dl	dl	-
Dihydrocaffeic acid-3-*O*-sulfate	4-OH	-	3-*O*-s		1656±1116d	8.1±3.1d	dgk	d	-
Dihydrocoumaric acid					-	-	l	l	-
Dihydrocoumaric acid-*O*-sulfate					-	-	l	l	-
Dihydro-*p*-coumaric acid	4-OH	-	-		-	-	i	i	-
Dihydroferulic acid	4-OH	3-OCH_3			304±122d	6.1±3.4d	dfjl	d	-
Dihydroferulic acid-4-*O*-glucuronide	-	3-OCH_3	4-*O*-glc		201±59d	7.1±3.3d	dg	d	-
Dihydroferulic acid-4-*O*-sulfate	-	3-OCH_3	4-*O*-s		197±96d	6.8±3.3d	dgl	dl	-
Dihydroisoferulic acid	3-OH	4-OCH_3	-		-	-	f	-	-
Dihydroisoferulic acid-3-*O*-glucuronide	-	4-OCH_3	3-*O*-glc		23±10d	5.1±2.5d	d	d	-
Dihydroisoferulic acid-3-*O*-sulfate	-	4-OCH_3	3-*O*-s		97±42d	4.0±2.5d	d	d	-
3-Hydroxyphenylpropionic acid	3-OH	-	-		-	-	fg	g	-
3-Hydroxyphenylpropionic acid sulfate	3-OH	-	-		-	-	g	-	-

Metabolite	Benzene ring substituents			Functional group in C1 of benzene	C_{max} (nM)	t_{max} (h)	Detected in:		
	OH	OCH_3	Others				Urine	Blood	Faeces
Pyrogallol derivatives									
Pyrogallol-1-*O*-sulfate	2,3-OH	-	1-*O*-s		199±79d	8.7±3.1d	d	d	-
Pyrogallol-2-*O*-sulfate	1,3-OH	-	2-*O*-s		339±123d	6.2±2.3d	d	d	-
1-Methylpyrogallol-*O*-sulfate	diOH	1-OCH_3	-*O*-s		538±172d	9.1±3.8d	d	d	-
2-Methylpyrogallol-*O*-sulfhate	diOH	2-OCH_3	-*O*-s		185±44d	5.3±2.6d	d	d	-
Phloroglucinol	1,3,5-OH	-	-		-	-	g	-	-

a, *n* = 8, 500 mg of ^{13}C-labelled cyanidin-3-*O*-glucoside, errors are given as standard error of the mean (SEM)[158]; **b**, *n* = 15, 500 mg of elderberry anthocyanins[183]; **c**, *n* = 52, 500 mg of elderberry anthocyanins[40]; **d,** *n* = 10, cranberry juice containing 787 mg of polyphenols of which 16.2 mg are anthocyanins[35]; **e**, *n* = 8, 500 mg of ^{13}C-labelled cyanidin 3-*O*-glucoside[159]; **f**, *n*=6, bilberry and lingonberry puree containing 500 mg of anthocyanins[32]; **g,** *n* = 2, 125 g of raspberries containing 71 mg of anthocyanins[37]; **h,** *n* = 5, 150 g bilberries containing 2.5 g of anthocyanins[34]; **i**, *n* = 6**,** 500 mg of aronia berry extract containing 45.1 mg of anthocyanins[33]; **j**, *n* = 5, 400 g of purple potatoes, amount of anthocyanins unknown[175]; **k**, *n* = 9, 300 g of blended raspberries, containing 553 µmol of polyphenols, of which 53% anthocyanins (292 µmol), 45% ellagic acid conjugates, 2% phenolic acids), and errors are given as standard errors (SE)[184]; **l**, (*n* = 8), 350 mL of 100% grape juice containing 528 µmol of polyphenols, of which 46% were anthocyanins, and of which 40% were petunidin derivatives acylated to acetic acid or *p*-coumaric acid[90]; **m**, *n* = 8, 350 mL of grape juice containing 528 µmol polyphenols of which 46% was partly acylated anthocyanins[155]; **n**, *n* = 10, 330 mL of grape/blueberry juice containing 278 mg of anthocyanins of which 55 mg were monoacylated (acetic acid and/or *p*-coumaric acid) malvidin, peonidin, petunidin, cyanidin and delphinidin glucosides[169]; **o**, *n* = 10, 330 mL of grape/blueberry smoothie containing 324 mg of anthocyanins of which 46 mg were monoacylated (acetic acid and/or *p*-coumaric acid) malvidin, peonidin, petunidin, cyanidin and delphinidin glucosides[169].

The metabolites of the study **m** were detected using GC-MS[155].

*t_{max} includes isomers due to analytical challenges related to chromatographic separation; **deviation not reported.

2.3 Effect of anthocyanin-rich foods on postprandial glycaemia and insulinaemia

2.3.1 Overview of postprandial glucose metabolism

Dietary carbohydrates, such as sucrose and starch, are important sources of energy. According to the Nordic Nutrition Recommendations, 45–60% of the daily energy intake should be acquired from carbohydrates, of which <10% may be simple sugars.[185] In human metabolism, digestible dietary carbohydrates are broken down into monosaccharides by several enzymes. Salivary and pancreatic α-amylases break down starch (α-1,4 glycosidic linkages) to di- and oligosaccharides, such as maltose, maltotriose and α-limit dextrins. Their decomposing is continued in the brush-border of small intestine enterocytes by disaccharidases, such as maltase (α-1,4 glycosidic linkages) and isomaltase (α-1,6 and α-1,4 glycosidic linkages). Other disaccharidases include sucrase (α-1,2 glycosidic links of sucrose), trehalase (α-1,1 glycosidic linkages of trehalose) and lactase (β-1,4 glycosidic linkages of lactose).[186]

Absorption of glucose and galactose to enterocytes occurs via SGLT1 and fructose via GLUT5. In the basolateral side of enterocytes, GLUT2 and GLUT5 direct the monosaccharides into the circulation system. Thereafter, fructose and galactose are converted to glucose. Absorbed glucose raises the blood glucose levels and induces the secretion of insulin from the Langerhans β-cells of the pancreas, required for the uptake of glucose to muscle and adipose tissues via GLUT4.[187] Glucose homeostasis is orchestrated strictly hormonally, and failure in the regulation may lead to severe complications, such as type II diabetes and metabolic disorders.

2.3.2 Pure anthocyanins

Studies reporting the effect of purified anthocyanins on postprandial blood glucose and insulin of human volunteers are limited. In two rodent model studies conducted with normoglycaemic and healthy rats and mice, an oral administration of cyanidin-3-*O*-rutinoside[188] and pelargonidin-3-*O*-rutinoside[189] decreased postprandial blood glucose as compared to the control group. These studies did not measure insulin levels in blood. In another study, the effect of the structural characteristics of different anthocyanidins and anthocyanins on the stimulation of insulin secretion was investigated *in vitro* in the pancreatic β-cells of rodents (INS-1 832/13). The number of hydroxygroups in the B-ring was found to be an essential factor for anthocyanins to stimulate the insulin secretion, as delphinidin-3-glucoside was the most efficient followed by cyanidin-3-glucoside and pelargonidin-3-galactoside. Methoxysubstituted anthocyanins

were not studied. However, only pelargonidin increased insulin secretion out of the five studied anthocyanidins (cyanidin, delphinidin, pelargonidin, malvidin and petunidin), indicating the potential role of the sugar moieties.[190] Purified non-acylated anthocyanins showed a long-term hypoglycaemic effect and increased insulin sensitivity in diabetic rodent models (cyanidin-3-*O*-glucoside)[191,192] and diabetic human volunteers (purified bilberry and blackcurrant anthocyanins).[193]

The effect of purified acylated anthocyanins on postprandial glycaemia has been studied with rodents. One dose of extracted peonidin-3-*O*-[2-*O*-(6-*O*-feruloyl-glucoside)-6-*O*-caffeoyl-glucoside]-5-*O*-glucoside from purple sweet potatoes (100 mg/kg) followed by maltose (2 g/kg) decreased the AUC120 min and both postprandial blood glucose and insulin at 30 and 60 minutes in normoglycaemic Sprague-Dawley rats (n = 4). Interestingly, ingesting the anthocyanin did not affect postprandial glucose and insulin when consumed with a dose of sucrose or glucose.[194] When hyperglycaemic C57BL/6 mice (n = 5) were given one dose (80 mg/kg) of diacylated cyanidin-3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside or peonidin-3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside extracted from purple sweet potatoes, the cyanidin derivative decreased the postprandial blood glucose one and two hours after the meal and peonidin derivative decreased the blood glucose levels one hour after the meal. Insulin was not, however, analysed.[195] Results of the studies on acylated anthocyanins are presented in **Table 4**. These studies provide evidence that both non-acylated and acylated anthocyanins affect the postprandial glycaemia of healthy and diabetic organisms beneficially. The potential mechanisms underlying these effects are discussed in Chapter 2.5.

2.3.3 Foods rich in non-acylated anthocyanins

Clinical trials show that foods rich in non-acylated anthocyanins, such as various berries, affect postprandial glycaemia and insulinaemia beneficially. Examples of foods showing acute postprandial hypoglycaemic effect on healthy human volunteers include blueberries[196], lingonberries[197], black currants (glucose and insulin)[198,199], black currant juice fortified with crowberry (glucose and insulin)[200] and berry mixtures (bilberries, black currants, cranberries, strawberries, lingonberries, or cranberries) (glucose and insulin except for reference[201])[201–203]. Some acute-phase studies report no effect on postprandial glycaemia in healthy volunteers after consuming blueberries and raspberries[204], raspberries, cloudberries and chokeberries.[203] Habitual high intake of anthocyanin-rich foods have been related to lowered insulin levels and insulin resistance, but not blood glucose, in normoglycaemic women (n = 1997).[205] Both

acute and chronic effects of foods rich in non-acylated anthocyanins on glycaemia are reviewed elsewhere in detail.[10,11,13,206–210]

2.3.4 Foods rich in acylated anthocyanins

Investigations of the effect of foods rich in acylated anthocyanins on postprandial glycaemia and insulinaemia are limited, and have been mainly conducted with animal models and as long-term studies. The studied foods include purple and red potatoes[211–216], purple sweet potatoes[194,217], purple carrots[212,218], red cabbage[219] purple water yam[220], red lettuce[96] and Concord grapes[154] (**Table 4**). The studies give evidence of the hypoglycaemic effect of these foods, but the bioactive compounds may not be deduced while using whole foods.

The effect of pigmented potatoes on glycaemia has been studied as cooked[211–213], lyophilised powder[214], chips[215] and polyphenolic extracts[216]. An acute dose of oven-baked purple (Purple Majesty) and red potatoes (Y38), the amount corresponding to 50 g of available carbohydrates (290–380 g of potato) with 16 and 15 mg/100 g DW of anthocyanins, decreased the postprandial glucose of healthy volunteers (n = 9) 15 min after intake compared to a control dose of glucose which had no effect on AUC120 min or insulin. Red potatoes also increased postprandial glucose at 120 min compared to the control dose of glucose.[211] A long-term study conducted with hypertensive and overweight/obese volunteers showed that 138 g of microwave-cooked purple potatoes (Purple Majesty) containing 276 mg of phenolic compounds per meal and 6.5 mg/g DW anthocyanins twice daily for four weeks did not alter fasting state glycaemia or glycated haemoglobin (HbA1c).[213]

When purple potatoes (Bora Valley) were sliced, lyophilised and fed daily (0%, 10% or 20% of the baseline diet) for seven weeks to diabetic rats (n = 8 in each group), the powder decreased the weekly measured fasting state glucose of the diabetic rats fed with 10% and 20% of purple potatoes at the third week, but not at weeks five and seven when compared to the diabetic control group. The fasting state insulin measured after the chronic intake was significantly increased in the diabetic group fed with 20% of purple potatoes compared to the control group of diabetic rats.[214] Furthermore, baked and freeze-dried purple potatoes (450 g/kg of diet, 1.47 mg/g DW of anthocyanins) or white potatoes (450 g/kg of diet) were fed to obese rats (n = 15 per group) for eight weeks with a high-fat diet, and the results were compared to a control group fed with the high-fat diet without potatoes. The chronic supplementation of purple potatoes decreased the blood glucose levels 60, 90 and 120 minutes after the glucose tolerance test when compared to the control and white potato groups. Blood glucose was also decreased 30 and 45 minutes after the insulin tolerance test when compared to

the control group. Fasting state insulin was significantly lower in the groups fed with the purple potatoes and white potatoes when compared to the control group. The purple potatoes also enhanced insulin sensitivity compared to the control group, measured in the intraperitoneal insulin tolerance test and homeostatic model assessment of insulin resistance (HOMA-IR).[212]

In a clinical trial, an acute intake of purple potato chips providing 50 g of available carbohydrates and 1.05 mg of anthocyanins caused a non-significant decreasing trend in the postprandial glucose response calculated as AUC120min and AUC30–60min compared with the red (3.1 mg of anthocyanins) and white potato chips. Both red and purple chips delayed the peak glucose concentration time. Insulin was not measured.[215] Furthermore, a purple potato extract rich in acylated anthocyanins (Blue Congo, 72.7 mg/g DW of anthocyanins) and phenolic acids (167.4 mg/g DW, caffeoylquinic acid derivatives) fed to diabetic rats for two weeks showed statistically insignificant decrease of fasting state glucose and significant decrease of glycated haemoglobin compared to the diabetic rats not fed with the extract. Insulin was not measured.[216]

Table 4. Effect of foods rich in acylated anthocyanins on the fasting state (fs) and postprandial (pp) glycaemia of rodents and human volunteers. In the case of animal studies, *n* represents the number of the animals per intervention group.

Food origin	*n*	Study design	Study duration	Control	Effect on blood glucose	Ref.
Purified acylated anthocyanins						
Purified anthocyanin[a] from purple sweet potato	4	Animal study with normoglycaemic rats	Acute oral administration	Maltose, sucrose or glucose without anthocyanins	Decreased pp glucose and insulin after maltose intake at 30 and 60 min. No effect on pp glucose or insulin after intake of sucrose and glucose Decreased AUC120 min	194
Purified anthocyanin[b] from purple sweet potato	5	Animal study with hyperglycaemic mice	Acute oral administration	Distilled water	Decreased pp glucose at 1 and 2 h	195
Purified anthocyanin[c] from purple sweet potato	5	Animal study with hyperglycaemic mice	Acute oral administration	Distilled water	Decreased pp glucose at 1 h	195
Pigmented potatoes rich in acylated anthocyanins						
Purple potatoes (freeze-dried, baked)	15	Animal study with obese rats	Daily oral intake for 8 weeks	High-fat (HFD) baseline diet or high-fat baseline diet + white potatoes (WP)	Decreased pp glucose at 60, 90 and 120 min during ipGTT compared to WP and HFD Decreased pp glucose at 45min during ipITT compared to HFD Purple potatoes and WP decreased fs insulin Enhanced insulin sensitivity compared to HFD	212
Purple potato (lyophilised powder)	8	Animal study with diabetic rats	Daily oral intake for 7 weeks	Baseline diet	Decreased fs glucose at week 3, no effect at weeks 5 and 7 Increased fs insulin	214

Food origin	*n*	Study design	Study duration	Control	Effect on blood glucose	Ref.
Purple potato[d] (dried phenolic extract)	8	Animal study with diabetic rats	Daily oral intake for 2 weeks	Baseline diet	Statistically insignificant decrease of fs glucose Decrease of HbA1c	216
Purple and red potatoes (oven-baked)	9	Clinical trial (randomized blocks, cross-over) with healthy human volunteers	Acute	Glucose or white potatoes or yellow potatoes	Decreased pp glucose at 15 min (all potato types versus control glucose) and increased pp glucose at 120 min after red potato meal versus control (glucose). No effect on pp insulin No effect on AUC 120 min of glucose or insulin	211
Purple and red potato (chips)	11	Clinical trial (cross-over) with healthy human volunteers	Acute	Salted wheat crackers or white potato chips	Decreased pp glucose compared to crackers Non-significant, decreasing trend of total AUC120 min and iAUC30–60 min of purple chips compared to other chips Delayed glucose peak time of both chips	215
Purple potatoes (microwave-cooked)	18	Clinical trial with hypertensive, overweight or obese volunteers	Oral intake twice daily for 4 weeks	Potato-free diet	No effect on fs glucose No effect on HbA1c	213
Other foods rich in acylated anthocyanins						
Purple sweet potato (anthocyanin extract)	4	Animal study with normoglycaemic rats	Acute oral administration	Maltose, sucrose or glucose without anthocyanins	Decreased pp glucose after maltose intake at 30 min and insulin at 30 and 60 min No effect on pp glucose after intake of sucrose and glucose	194
Water yam (extract)	5	Animal study with hyperglycaemic rats	Daily oral dose for 4 weeks	Baseline diet	Decreasing trend of fs glucose each week 1–4	220

Food origin	*n*	Study design	Study duration	Control	Effect on blood glucose	Ref.
Concord grape supplement[g]	8	Animal study with obese rats	Daily oral dose for 12 weeks	Baseline diet	No effect on fs glucose No effect on glucose during OGTT or ipGTT	154
Purple carrots (raw)	15	Animal study with obese rats	Daily intake for 8 weeks	Baseline diet and baseline diet + orange carrots	No effect on glucose Non-significant decrease of fs insulin after purple carrots compared with orange ones and baseline	212
Red lettuce[f] (lyophilised leaf powder)	8	Animal study with obese, insulin resistant hyperglycaemic mice	Daily intragastric dose for 8 days	Baseline diet + vehicle (water)	Decreased pp glucose measured 6 h after administration on the sixth treatment day Decreased pp glucose at 30 minutes during OGTT after 7 treatment days Improved insulin response in insulin tolerance test	96
Purple sweet potato extract (lyophilised)	10	Animal study with diabetic mice	Daily oral dose for 5 weeks	Baseline diet	Decreased fs glucose	217
Purple carrot (pasteurised juice)	12	Animal study with diabetic mice	Daily oral dose for 8 weeks	Baseline diet	Decreased glucose during OGTT (AUC120 min)	218
Red cabbage[e] (lyophilised extract)	8	Animal study with diabetic rats	Daily intragastric dose for 4 weeks	Baseline diet + saline	Decreased fs glucose Decreased HbA1c Lowered glucose during OGTT (AUC60 min)	219

ANC, anthocyanin; NA, not available; pp, postprandial; AUC, area under curve; ipGTT, intraperitoneal glucose tolerance test; ipITT, intraperitoneal insulin tolerance test; OGTT, oral glucose tolerance test; HbA1c, glycated haemoglobin.

Main reported anthocyanins in the foods: **a**, peonidin-3-*O*-[2-*O*-(6-*O*-feruloyl-glucopyranosyl)-6-*O*-caffeoyl-glucopyranoside]-5-*O*-glucopyranoside; **b**, cyanidin-3-caffeoyl-p-hydroxybenzoylsophoroside-5-glucoside; **c**, peonidin-3-(6″-caffeoyl-6‴-feruloylsophoroside)-5-glucoside; **d**, petunidin-3-*O*-*p*-coumaroyl-rutinoside-5-*O*-glucoside; **e**, delphinidin-3-rutinoside-5-hexoside + cyanidin-3-caffeoylferuloylsophoroside-5-glucoside + delphinidin-3-feruloyl-rhamnosyl-hexoside; **f**, cyanidin-3-malonyl-glucoside; **g**, delphinidin- and cyanidin-3-*O*-glucosides, delphinidin-3,5-*O*-(coumaroyl)diglucoside.

2.4 Effect of anthocyanins on inflammation markers

Consuming food, such as glucose and fat, evokes an acute, but short-timed, immunal response mediated by the innate immunity system which is linked with oxidative stress.[1,221–224] In healthy normoglycaemic volunteers, one meal high in carbohydrates or fat is enough to cause an inflammatory response with more severe effect from carbohydrates than fat.[225] The inflammatory response is dose-dependent; the higher the glycaemic index, the higher the inflammation response.[226] The acute postprandial inflammation is usually resolved quickly, but failure of resolvation leads to chronic inflammation.[223,224] Chronic inflammation ultimately impairs the function of the insulin-secreting pancreatic β-cells which further promotes the formation of obesity, insulin resistance and type 2 diabetes.[227–229]

Excessive flux of nutrients to the mitochondria overloads the mechanism for glucose decomposition, the tricarboxylic acid cycle, in muscle and adipose tissues. The overloaded tricarboxylic acid cycle produces excess amounts of nicotinamide adenine dinucleotide (NADH), all of which may not be reduced. Thus, the electrons are transferred to the oxygen, which leads to the formation of superoxide anion and other free radicals. In addition, nitrogen oxide and peroxy nitrate are formed. This state is called oxidative stress.[230] Meal-induced oxidative stress alters the insulin-dependent signalling pathways[229] and nuclear factor κB (NF-κB),[231,232] which leads to secretion of cytokines, such as interleukin-6 (IL-6) and the tumour necrosis factor α (TNF-α).[225,233] Oxidative stress is prolonged in obesity compared to a healthy state[231] and high, repetitive postprandial glucose fluctuations cause more oxidative stress in both healthy and diabetic volunteers than chronically elevated blood glucose.[4,234]

Inflammation status is investigated by analysing inflammation biomarkers. These biomarkers include, for example, lipid mediators, such as leukotrienes and prostaglandins, and soluble peptides, such as cytokines, chemokines and adhesion markers.[235] Calder et al. also suggested that instead of measuring the exact concentration of a certain biomarker, it could be more useful to measure concentration changes and biomarker patterns.[235]

Improving antioxidant defence mechanisms prevents oxidative stress and decreases postprandial inflammation. Dietary antioxidants include for example carotenoids, vitamins E and C and polyphenolic compounds, such as flavonoids.[223] Anthocyanins and anthocyanin-rich foods have been shown to alter inflammation markers (**Table 5**), but in the case of whole foods, the bioactivity may not be unambiguously linked to the anthocyanins. The acute postprandial effect of anthocyanin-rich foods on healthy volunteers have been seen to decrease CRP and IL-6 with strawberries[236], IL-4 with cranberry juice[237] and MCP-1 with red wine anthocyanins[238] but not with black currant juice[239]. In

overweight men, a 4-day daily intake of black raspberries reduced postprandial IL-6 after an acute intake of black raspberries with a meal.[240] Another study investigating the acute postprandial effect of strawberries with overweight volunteers did not find that the berries altered the inflammation markers with or without a six-week chronic intake.[241] Furthermore, a chronic daily supplement of purified anthocyanins from bilberries and black currants increased fasting state IL-8 (CXCL8), IFNα and RANTES (CCL5)[242] and decreased fasting state IL-10 (negative dose-dependence), IL-6[243] in healthy volunteers, and fasting state IL-6 and TNF-α in diabetics[193]. In hypercholesterolemic individuals, anthocyanins of bilberries and black currants decreased fasting state sVCAM-1 (soluble vascular adhesion molecule-1)[244] and fasting state hsCRP, IL-1β and platelet-derived chemokines CXCL5, CXCL7, CXCL8 (IL-8), CXCL12 and CCL2.[245] The effect of the chronic intake of different berries on the fasting state inflammation markers has recently been reviewed elsewhere.[246,247]

The current literature is, again, limited about the effect of acylated anthocyanins on the inflammation biomarkers in the human postprandial state. Long-term effects were investigated in the study by Kaspar et al. in which three groups of 12 healthy volunteers consumed 150 g of white, yellow or purple potatoes daily for six weeks. Fasting state CRP was reduced in the purple potato group as compared to the white potato group, whereas IL-6 was reduced by both red and purple potato treatments in comparison with the white potatoes.[248] *In vitro*, Zhang et al. showed that a semi-purified purple potato extract rich in petunidin-3-*O*-*p*-coumaroylrutinoside-5-*O*-glucoside and a purple carrot extract added to TNF-α stimulated Caco-2 cell monolayer decreased IL-8 levels. The absorbed purple carrot and purple potato anthocyanins inhibited the expression of inflammatory cytokines TNF-α and IL-8 in the lipopolysaccharide-induced macrophages (THP-1) both at protein and gene levels, but the purple potato was more efficient than the purple carrot, in a co-culture with Caco-2 BBe1 cells.[147]

Table 5. Known biological functions of the soluble inflammation biomarkers which have been modified by acute or chronic intake of anthocyanin-rich foods in recent literature. The alternative name, when applicable, is in brackets.

Biomarker of inflammation	Abbr.	Biological function
Acute phase proteins		
C-reactive protein	CRP	Clearing dead cells and pathogens via opsonisation and activating complement, and production of cytokines[235,249]
CC Chemokines		
Chemokine (C-C motif) ligand 2 (monocyte chemoattractant protein-1)	CCL2 (MCP-1)	Recruits leucocytes such as monocytes, dendritic cells and memory cells[235,250]
Chemokine (C-C motif) ligand 5 (regulated on activation, normal T expressed and secreted)	CCL5, (RANTES)	Proinflammatory, recruits leucocytes such as T cells, basophils and eosinophils, activates natural killer cells[235,251]
CXC Chemokines		
Chemokine (C-X-C motif) ligand 5	CXCL5	Induces neutrophil chemotaxis[252]
Chemokine (C-X-C motif) ligand 7	CXCL7	Induces neutrophil chemotaxis[252]
Chemokine (C-X-C motif) ligand 8	CXCL8 (IL-8)	Mediates innate immune response by triggering neutrophil infiltration[253]
Chemokine (C-X-C motif) ligand 12	CXCL12	Recruits leucocytes[252,254]
Other chemokines and cytokines		
Interferon alpha	IFN-α	Modulates immune responses, confers non-specific resistance to viral infections and affects cell proliferation[255]
Interleukin-4	IL-4	Differentiates naive CD4 T cells into T_h2 cells and controls the class of the produced immunoglobulin in the course of an antibody response[256]
Interleukin-6	IL-6	Chemokine; activates neutrophils and lymphocytes, IL-1ra and IL-10, inhibits TNF and IL-1, [235], macrophage activation, production of acute phase proteins like CRP[257]
Interleukin-10	IL-10	Anti-inflammatory cytokine: decreases expression of proinflammatory cytokines, improves B-cell survival and antibody production[235,258]
Tumour necrosis factor alpha	TNF-α	Induces synthesis of acute-phase proteins, T_h-cell differentiation and apoptosis[235]

2.5 Underlying molecular mechanisms behind the physiological effects of anthocyanins

The evidence of the glycaemia and inflammation lowering effect of anthocyanins raises a question about the underlying biochemical mechanisms. This leads to scrutinizing the connection between anthocyanins and the regulators of glucose homeostasis, such as the enzymatic digestion of carbohydrates, the uptake of glucose to target tissues and the multifaceted regulation of the secretion of the endocrine hormones of pancreas. Some of these mechanisms are briefly reviewed here with the emphasis on pure anthocyanins and acylated anthocyanins whenever possible. The effect of whole berries and berry extracts rich in non-acylated anthocyanins is reviewed elsewhere.[11,207,259,260] As anthocyanins have low bioavailability but are degraded to phenolic metabolites, the effect of phenolic metabolites is also briefly reviewed.

Inhibition of digestive enzymes. Several studies conducted with cell models indicate that anthocyanins inhibit the enzymes digesting carbohydrates as the IC_{50} values, which measure the inhibitory activity, were within micromolar range. Inhibitory activity shows structure-dependence. Starting from the human salivary α-amylase, it was shown to be inhibited *in vitro* by cyanidin glucoside, and by cyanidin > delphinidin > delphinidin glucoside > malvidin > cyanidin arabinoside in descending order of inhibitory activity. Petunidin and peonidin were not studied. The phenolic metabolites of anthocyanins were shown to inhibit human salivary α-amylase even more than the hydroxysubstituted non-acylated anthocyanins with descending order of 4-hydroxybenzaldehyde > gallic acid > protocatechuic aldehyde > ferulic acid > protocatechuic acid > syringic acid > vanillic acid.[261]

Considering pancreatic α-amylase, the pancreatic α-amylase from pigs was inhibited by cyanidin and cyanidin glucoside but not by cyanidin galactoside or cyanidin-3,5-diglucoside.[262] Cyanidin-3-sambubioside inhibited pancreatic α-amylase from pigs.[263] Comparison of different anthocyanin glucosides shows inhibition of pancreatic α-amylase from pigs with non-significant small differences (pelargonidin glucoside > malvidin glucoside > cyanidin glucoside > delphinidin glucoside > peonidin glucoside). Again, petunidin glucoside was not studied. On the other hand, anthocyanins acylated with hydroxycinnamic acids, originating from black carrots, were demonstrated to inhibit pancreatic α-amylase even more when compared to the non-acylated ones.[264] In the case of pigmented potatoes, the literature is not unanimous. Moser et al. report that the phenolic extracts from purple and red potatoes did not inhibit pancreatic α-amylase from pigs *in vitro*[215] whereas Kalita et al. report that the phenolic extracts from purple, red, yellow and white potatoes inhibited pancreatic α-amylase, but the purple and red potatoes were more efficient inhibitors than

the yellow and white varieties.[265] The effect of the phenolic metabolites of anthocyanins on pancreatic α-amylase is, however, modest compared to cyanidin glucoside and sambubioside (protocatechuic acid > phloroglucinaldehyde > caffeic acid > 4-hydroxybenzaldehyde > ferulic acid > vanillic acid > *p*-coumaric acid > homovanillic acid > 4-hydroxybenzoic acid > hippuric acid).[266]

Continuing on to the effect of anthocyanins on the intestinal α-glucosidase, the brush-border enzyme of the small intestine with sucrase and maltase activities, a structure-dependent inhibition has been detected. Xu et al. studied the inhibitory effect of 18 isolated anthocyanins on α-glucosidase. Of these, pelargonidin glucoside inhibited α-glucosidase *in vitro* the most. The inhibitory activity of anthocyanidins followed the order of pelargonidin > malvidin > peonidin > delphinidin > petunidin > cyanidin, and for glycosides, rutinosides > glucosides and arabinoside > glucoside > galactoside.[189] Inactivity of cyanidin and its glycosides towards maltase was detected also in other studies[262,263], whereas cyanidin rutinoside slightly inhibited rat intestinal maltase[188] and cyanidin sambubioside actively (yeast α-glucosidase and porcine pancreas maltase)[263]. Acylation, again, seems to increase the inhibitory activity of the anthocyanin on α-glucosidase. Two diacylated purple sweet potato anthocyanins, cyanidin- and peonidin-3-*O*-[2-*O*-(6-*O*-feruloyl-glucose)-6-*O*-caffeoyl-glucose]-5-*O*-glucose), were shown to strongly inhibit the maltase activity of rat intestinal α-glucosidase, but they did not inhibit the sucrase activity. Acylation to caffeic and ferulic acid was essential for the inhibitory activity towards maltase as deacylation drastically decreased the inhibitory efficiency.[267] Furthermore, rats fed with sucrose, glucose or maltose and a diacylated anthocyanin from purple sweet potato (peonidin-3-*O*-[2-*O*-(6-*O*-feruloyl-glucose)-6-*O*-caffeoyl-glucose]-5-*O*-glucose) showed suppressed glycaemia and reduced serum insulin only in the case of maltose, implying that the hypoglycaemic effect of the diacylated anthocyanin was related to inhibiting the maltase activity.[194] Intestinal sucrase of rats was inhibited only modestly by cyanidin and its glycosides (cyanidin galactoside > cyanidin glucoside > cyanidin), whereas cyanidin-3,5-diglucoside did not inhibit sucrase at all.[262] Other studies reported that cyanidin and cyanidin-3-glucoside did not inhibit sucrase[263] whereas cyanidin-3-rutinoside[188] and cyanidin-3-sambubioside[263] did. Kalita et al. showed that the phenolic extract of purple potatoes inhibited α-glucosidase more than those of the red, yellow and white cultivars.[265] However, in a study by Moser et al., two purple potato cultivars and one white potato cultivar, but not the red cultivars, had a modest inhibitory effect on rat intestinal α-glucosidase *in vitro.*[215] Phenolic metabolites (protocatechuic acid, gallic acid, vanillic acid, protocatechuic aldehyde, ferulic acid, 4-hydroxybenzaldehyde, syringic acid and chlorogenic acid) did not inhibit yeast α-glucosidase statistically significantly[261] but inhibited intestinal α-glucosidase (protocatechuic

acid > phloroglucinaldehyde > caffeic acid > ferulic acid > 4-hydroxybenzaldehyde > vanillic acid > *p*-coumaric acid > homovanillic acid > 4-hydroxybenzoic acid > hippuric acid).[266]

Inhibition of absorption of glucose. After the dietary carbohydrates are decomposed to monosaccharides by the digestive enzymes, glucose is transported to the enterocytes of the small intestine, and from there, to the systemic circulation. Anthocyanins have been shown to inhibit the absorption of glucose *in vitro* structure-dependently. In a study by Barik et al., cyanidin was the most effective in inhibiting the uptake of glucose followed by delphinidin > malvidin > cyanidin-3-glucoside > delphinidin-3-glucoside as investigated with a human intestinal cell model, the Caco-2 cell monolayer.[261] In addition to cyanidin-3-glucoside, cyanidin-3-rutinoside decreased the uptake of glucose using rat jejunums *ex vivo*.[142] Faria et al. showed that pretreating the Caco-2 cell monolayer for four days with a grape anthocyanin extract rich in malvidin-3-glucoside, resulted in decreased uptake of glucose.[144] In studies of Hidalgo et al., delphinidin and maqui berry extract rich in delphinidins reduced the uptake of glucose in the mouse jejunal mucosa *ex vivo* in the Ussing chamber.[268,269] Both the semi-purified extracts of purple potatoes and purple carrots rich in acylated anthocyanins decreased the intestinal glucose uptake.[147] Moser et al. showed that the phenolic extracts of purple, red and white potatoes decreased the glucose uptake across the Caco-2 cell monolayer *in vitro*.[215] Barik et al. detected that some phenolic metabolites of anthocyanins inhibited the absorption of glucose across the Caco-2 cell monolayer (4-hydroxybenzaldehyde > gallic acid > ferulic acid > syringic acid > chlorogenic acid), and 4-hydroxybenzaldehyde, gallic acid and ferulic acid were more efficient than the anthocyanins mentioned above.[261]

The suggested molecular mechanisms for the reduced absorption of glucose are not unambiguous. As anthocyanins and glucose are taken up by the same transporters, competitive inhibition via steric hindrance has been suggested.[144,147,188,207,268] Downregulation of the gene transcription of the glucose and fructose transporters (SGLT1, GLUT2, GLUT5) may be another mechanism, however Barik et al. reported that the selected, but unnamed, anthocyanins and anthocyanidins did not have an effect. On the contrary, the phenolic metabolites of anthocyanins were shown to downregulate the transcription of the genes of SGLT1 (chlorogenic acid > gallic acid > syringic acid > 4-hydroxybenzaldehyde > vanillic acid), GLUT2 (chlorogenic acid > gallic acid > 4-hydroxybenzaldehyde > vanillic acid > ferulic acid > protocatechuic acid) and GLUT5 (protocatechuic aldehyde > ferulic acid).[261] Furthermore, delphinidin inhibited the intestinal glucose absorption by activating the free fatty acid receptor 1 (FFA1/GPR40), which leads to increased cAMP and cytosolic Ca^{+} oscillations.[269] GPR40 is known to stimulate insulin secretion.[270] Anthocyanidin glycosides, however, did not activate FFA1.[269,271]

Increased uptake of glucose into muscle and adipose tissues. Delphinidin-3-sambubioside-5-glucoside was detected to increase the uptake of glucose in rat L6 muscle cells.[272] Cyanidin, cyanidin-3-glucoside, cyanidin-3-sambubioside and phenolic metabolites (caffeic acid, *p*-coumaric acid, ferulic acid, phloroglucinaldehyde, 4-hydroxybenzaldehyde and vanillic acid) enhanced the uptake into human myotubes *in vitro*.[266] The possible underlying mechanism is to enhance the expression of GLUT4 by anthocyanins. GLUT4 is an insulin-dependent glucose transporter in the muscle and adipose tissues, and increased expression and translocation of GLUT4 to the cell surfaces leads to increased uptake of glucose in the tissues from circulation.[273] Indeed, cyanidin rutinoside increased GLUT4 expression and glucose uptake in human adipocyte cell model *in vitro*.[274] Cyanidin glucoside and its phenolic metabolite, protocatechuic acid, were shown to increase the uptake of glucose into human omental and murine adipocytes with and without stimulating the cells with insulin by enhancing the expression and translocation of the GLUT4 glucose transporters.[275] Furthermore, the increased expression of GLUT4 due to the effect of chronic intake of anthocyanins has been seen in the adipose tissues collected from sacrificed diabetic mice fed with cyanidin glucoside.[191]

The enhanced uptake of glucose into muscle and adipose tissues may occur via phosphorylation (activation) of adenosine monophosphate (AMP) activated protein kinase (AMPK). AMP-activated protein kinase cascade regulates the catabolic and anabolic energy metabolism reaction pathways.[276] Activation of AMPK leads to, for example, enhanced uptake of glucose via increased translocation of GLUT4 from the cytosol to the cell membrane.[277] Indeed, cyanidin glucoside activated the AMPK leading to enhanced expression of GLUT4 and the uptake of glucose in muscle model cells (rat L6 myotubes) *in vitro*.[278] In an *in vivo* study, after chronic consumption of blueberry anthocyanins by diabetic rats, AMPK was activated and expression of GLUT4 was increased in skeletal muscle and white adipose tissue of the sacrificed rodents.[279]

Increased uptake of glucose to adipocytes by GLUT4 may occur also by activating the insulin signalling pathway, the PI3K/Akt pathway. Cyanidin rutinoside was seen to increase the uptake of glucose into adipocyte model cells (3T3-L1) via PI3K/Akt pathway by phosphorylating, or activating, IRS-1 and Akt, and increasing the expression of PI3K. Interestingly, cyanidin rutinoside did not activate AMPK.[274] Protocatechuic acid, the well-recognised phenolic metabolite of anthocyanins, may increase the uptake of glucose and translocation of GLUT4 by interacting in an undefined way with the insulin receptor and affecting the insulin signalling pathway as it activates IRS-1 and Akt in human visceral adipocytes *in vitro*. When IRS-1, Akt and and PI3K were specifically inhibited, uptake of glucose and translocation of GLUT4 did not occur. Protocatechuic acid also activated AMPK.[280]

Decreased production of proinflammatory adipocytokines in adipocytes. In adipose tissue, the expression of GLUT4 may also be enhanced via adipocytokines. Adipocytes, in addition to being an energy storage, secrete biologically active adipocytokines, such as leptin and adiponectin. Adiponectins have antidiabetic properties; for example, they activate the insulin signalling pathway via PI-3K and AMPK of skeletal muscle and hepatocytes, and increase the uptake of glucose in muscle cells and suppress gluconeogenesis in the liver.[281] Interestingly, cyanidin, but not cyanidin glucoside, was shown to enhance the secretion of leptin and adiponectin in adipocytes collected from rats and treated *ex vivo*. AMPK activation was suggested as the molecular mechanism.[282] *In vivo*, the gene expression of adiponectin was upregulated after a 12-week administration of a purple corn extract rich in cyanidin glucoside also containing peonidin and cyanidin glucosides acylated with a malonic acid.[283] Cyanidin glucoside and protocatechuic acid increased adiponectin levels in human omental adipocytes and 3T3-L1 adipocytes via upregulating the gene transcription of PPARγ (peroxisome proliferator-activated receptor gamma), leading to increased translocation of GLUT4 to the membranes.[275] The PPARγ is a member of the nuclear receptor superfamily, which amongst other roles, regulates lipid and glucose homeostasis, expression of GLUT4 and expression of adipose tissue molecules such as adiponectin, resistin, leptin and TNF-α (tumour necrosis factor-α).[284,285] Moreover, the inflammatory adipocytokines MCP-1 (monocyte chemoattractant protein-1) and TNF-α (tumour necrosis factor-α) are present in obese and diabetic state and downregulate GLUT4. These adipocytokines were decreased after the chronic intake of cyanidin glucoside in diabetic mice. It was also suggested that this may further decrease the expression of another adipocytokine, RBP-4 (retinol-binding protein 4).[191] RBP-4 is known to interfere with insulin signalling in muscle tissue and to contribute to insulin resistance in obese and diabetic states.[286] In another study, blueberry anthocyanins downregulated RBP4 in diabetic rats.[279]

Inhibition of hepatic glucose production (gluconeogenesis). *In vitro*, glucose production was decreased in the hepatic cell model, HepG2, by cyanidin-caffeoyl-*p*-hydroxybenzoyl-sophoroside-5-glucoside and peonidin-3-caffeoyl-sophoroside-5-glucoside, but not with peonidin-3-(6″-caffeoyl-6‴-feruloyl-sophoroside)-5-glucoside, all extracted from purple sweet potatoes.[195] Cyanidin-malonyl-glucoside extracted from red lettuce did not inhibit the hepatic glucose production in the H4IIE rat hepatoma cells *in vitro*, but the red lettuce extract rich in phenolic compounds and aforementioned acylated anthocyanin did.[96] Hepatic gluconeogenesis may be inhibited via inhibiting the gluconeogenic enzymes with activation of hepatic AMPK. This was detected after six weeks of daily oral administration of black soybean seed coat extract and an eight-week administration of purple corn extract, both rich in non-acylated anthocyanins, to

diabetic mice: the hepatic AMPK was activated, which in turn lead to decreasing the rate-limiting gluconeogenic enzymes, phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6P).[278,283] The non-acylated, purified delphinidin-3-sambubioside-5-glucoside and the maqui berry anthocyanins decreased production of glucose in rat hepatoma cells modelling type II diabetes (H4IIE) *in vitro*. Additionally, the maqui berry anthocyanins downregulated G6P in the presence of insulin.[272] Decrease of the expression of G6P and PEPCK due to the decreased activation of the hepatic AMPK was also reported in the studies conducted with an anthocyanin-rich purple corn extract also containing malonylated cyanidin and peonidin glucosides[283], and blueberry extract[279]. Furthermore, the long-term fasting glycaemia may be improved via the Akt-mediated activation of FoxO1, as an eight-week daily oral administration of cyanidin-3-glucoside to diabetic mice resulted in its inhibition, leading to decreased G6P and PEPCK and decreased fasting state blood glucose.[192]

Enhancing secretion of insulin via increased incretin secretion. An acute meal of black currant juice increased the postprandial glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP) in healthy volunteers.[198] Both GLP-1 and GIP-1 are incretin hormones stimulating the secretion of insulin glucose-dependently. Kato et al. studied how six pure anthocyanidins and their glucosides, rutinosides of cyanidin and peonidin, and diglucoside of malvidin affect the secretion of GLP-1 in the murine enteroendocrine GLUTag L cell line *in vitro*. Delphinidin-3-rutinoside, delphinidin and malvidin were seen to stimulate the production of GLP-1. In the intestinal L cells, cytosolic Ca^{2+} was released and CAMKII (calmodulin-dependent kinase II) was activated, possibly via a G-protein coupled receptor GPR40/120.[271]

Glycaemic index. Food with a high glycaemic index initiate a more severe inflammatory response compared to foods with lower glycaemic index.[226] Potatoes have generally high glycaemic index regardless of the variety and cooking method.[42] Ramdath et al. studied the glycaemic indices of purple, red, yellow and white potato varieties, and no statistically significant differences were found. However, a significant inverse correlation between the glycaemic indices and polyphenols was found.[211]

Radical scavenging capacity. In the postprandial state, anthocyanins may contribute to the increased postprandial plasma antioxidant capacity in healthy volunteers after an acute meal of anthocyanin-rich berries, such as blueberries, mixed grapes and kiwifruit[287], tart cherries[288], acai berry[289] strawberries[236,290], blueberries[179,291], black currants[292], but not with cranberry juice[237], black currant juice[239] or anthocyanin-rich red wine extract[238]. A meal of purple potatoes, however, did not increase the plasma antioxidant capacity of healthy volunteers[211,215] but did in a mixed study population of normal weight (n = 5), overweight (n = 2) and obese (n = 1) volunteers[213]. Serum anthocyanin content

is positively correlated with the postprandial antioxidant status.[179] Three *in vitro* studies in which purple potatoes, purple sweet potatoes and red cabbage rich in acylated anthocyanins were incubated in a gastrointestinal model suggest that the antioxidant capacity is due to the intact gastric acylated anthocyanins and their subsequent phenolic degradation products.[133–135] Similarly, an acute meal of purple potatoes raised the plasma antioxidant capacity first at 30 min and then at 2 h, and further increased it towards the last sampling point, from 4 h to 8 h.[213]

Anthocyanins may protect from oxidative stress as they have a planar, delocalised, π-conjugated ring system which enables their strong antioxidant properties via scavenging different free radicals, such as DPPH (2,2-diphenyl-1-picrylhydrazyl), ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) and OH radicals, by donating hydrogens and electrons, and via the resonance effect of the aromatic nucleus. Considering the structure-antioxidant activity relationships, the importance of *o*-dihydroxystructure of cyanidin and trihydroxylation of delphinidin, the increased number of hydroxygroups in the B-ring, and a free 3-OH in the C-ring, are required for the most efficient scavenging activity. Mono- and dimethoxygroups decrease the activity, as do glycosylation.[293–296] According to Kähkönen et al., the radical scavenging efficiency of the glycosyl groups descended in the order of glucose > galactose > diglucose. The only studied acylated anthocyanin, cyanidin-3-coumaroyl-xyloside-glucoside)-5-galactoside, showed similar radical (DPPH) scavenging capacity as the anthocyanin monoglucosides.[295] Diacylated anthocyanins from purple sweet potatoes[195,239] and monoacylated nasunin from aubergines[297] also showed radical scavenging and reducing activity *in vitro*. At pH 7.4, both mono- and diacylation with hydroxycinnamic acids increased the radical scavenging capacity (ORAC) in cyanidin glycosides[22] and monoacylation with *p*-coumaric acid increased the antioxidative activity of malvidin glycosides measured by linoleic acid oxidation.[298] As regards purple potatoes, their double-triple increase in antioxidant activity as compared to white potatoes[48] is related to their anthocyanin concentration[45,47]. Furthermore, purple potatoes show antioxidant activity even as snack products.[299,300] An anthocyanin-rich extract prepared from cooked purple potatoes showed a slightly decreased but still strong antioxidant activity when compared to the non-cooked sample as measured with a DPPH test after administrating to an *in vitro* gastrointestinal model.[301] However, comparing the results between different studies and different anthocyanin structures, including aromatic acylation, is challenging, due to dissimilar methods, solvent systems, pH, concentrations of anthocyanins and purity of the anthocyanins, and drawing conclusions on the basis of *in vitro* models may be misleading.

Enhanced endogenous antioxidant defence via Nrf2, NF-κB and MAPK. Acute consumption of anthocyanin-rich foods may increase the endogenous antioxidative defence systems, however this has received little attention in the

research. The existing literature suggest a late response; for example, acute consumption of cranberry juice rich in non-acylated anthocyanins increased reduced glutathione (GSH) and superoxide dismutase (SOD) 24 h after the meal in healthy volunteers.[237] Another study showed no effect on plasma glutathione within eight hours after an acute intake of bilberry extract rich in non-acylated anthocyanins in healthy volunteers[302], suggesting a later effect possibly due to the phenolic metabolites. However, transcription of NAD(P)H dehydrogenase (NQO1) was increased and transcription of heme oxygenase 1 (HO-1) was decreased. The suggested underlying molecular mechanism behind the activated defence mechanisms may involve Nrf2, the regulator of NQO1 and HO-1, as its transcription was also increased.[302] Nrf2 is, indeed, an anti-inflammatory, redox-sensitive transcription factor which binds to the promoter areas called the antioxidant response element (ARE) inducing *e.g.* transcription of antioxidative enzymes (glutathione peroxidase GPx, glutathione reductase GR) and detoxifying enzymes (glutathione-S-transferase GST).[303] It was suggested that the bioactive compound was phloroglucinaldehyde, a common anthocyanin degradation product, as the anthocyanins and other studied phenolic metabolites (protocatechuic acid, syringic acid, vanillic acid, gallic acid, 4-hydroxybenzoic acid and benzoic acid) did not induce ARE promoter activity in an further *in vitro* study.[302] Chronic administration of foods rich in acylated anthocyanins to rats resulted in increased activity of SOD and GPx (purple potato flakes)[304], SOD, CAT, GPx, GSH (purple potato extract)[216] and GPx and CAT (red cabbage extract)[219]. *In vitro*, transcription of Nrf2 is known to be enhanced by cyanidin-3-glucoside.[305] Pure anthocyanins and anthocyanidins were shown to upregulate the ARE-regulated phase II antioxidative enzymes (GR, GPx, GST) *in vitro* in a rat hepatocyte Clone 9 cell line. Cyanidin, cyanidin-3-glucoside, delphinidin and malvidin showed the best efficiencies amongst the most common six anthocyanidins and their glycosides (glucosides of petunidin and delphinidin were not studied).[306]

Nuclear factor-κB (NF-κB) is a central mediator of the human immune response, which is via activation responsible for the transcription of a number of genes, including those encoding cytokines, chemokines, immunoreceptors, cell adhesion molecules, acute phase proteins, growth factors and enzymes, such as superoxide dismutase (SOD), pro-inflammatory enzymes cyclo-oxygenase-1 (COX-2) and inducible nitric oxide synthase (iNOS).[233,307] Pure cyanidin-3-glucoside inhibits the NF-κB signal pathway in TNF-α exposed human intestine Caco-2 cells.[305] Purified anthocyanins from bilberries and black currants suppressed the activation of lipopolysaccharide-induced NF-κB and proinflammatory chemokine expression in monocytes.[242] Purple carrots rich in acylated anthocyanins decreased COX-2 and iNOS[308], and purple sweet potato extract lowered COX-2 *in vitro*[309].

MAPK (mitogen-activated protein kinases) are inflammation and cytokine stimulated kinases regulating *e.g.* cell differentiation, mitosis and apoptosis. Examples of MAPK's are ERKs, c-JNKs and p38 MAPKs.[310] Absorbed acylated anthocyanins of purple potatoes and purple carrots were shown to suppress lipopolysaccharide-induced phosphorylation of JNK and IkBα, indicating both MAPK and NF-κB pathways, in mucosal innate immune cells *in vitro*. Purple potato anthocyanins were more efficient and restored the expressions to the level of normal control cells.[147]

2.6 Concluding remarks and future prospects

Ingestion of food results in a postprandial state, leading to complex and dynamic metabolic processes involving various biochemical reactions, enzymes and endocrine signals and almost all organs and tissues, aimed at exploiting the macro- and micronutrients of food for survival and maintaining homeostasis. The postprandial state may cover the majority of a day, and therefore dietary choices and their impact on the postprandial state have the utmost importance on human health. Therefore, the focus of this literature review is on anthocyanin-rich foods, which have shown to have potential health effects in the postprandial state.

Pigmented vegetables and root vegetables provide another anthocyanin-rich source in addition to berries and fruits. Here, purple potatoes were chosen as a model food as potatoes are widely consumed world-wide but have a high glycaemic index. They contain structurally distinct anthocyanins as they are monoacylated with hydroxycinnamic acids. When considering the generally poor stability of anthocyanins, acylation with one or more aromatic and aliphatic acids increases the stability, which makes acylated anthocyanins a compelling natural food colourant for the food industry. Considering the bioavailability, several animal and clinical studies show notoriously poor uptake of anthocyanins in general, and it is even lower for the acylated anthocyanins. The metabolites of anthocyanins may have beneficial effects on human health in addition to the parent compounds; however, metabolism and health effects of acylated anthocyanins *in vivo* are poorly understood.

Postprandial metabolism of anthocyanins is, in its entirety, a highly complex multiorgan reaction chain starting from the mouth and reaching the gut microbiota, involving several enzymes, xenobiotic metabolism and enterohepatic circulation. Summing up the current literature, anthocyanin structures, such as acylation, affect the postprandial stability, absorption, metabolism, glycaemic effects and antioxidative properties of anthocyanins. *In vitro* studies reveal that acylation stabilises anthocyanins both in the mouth and in gastric conditions. Acylated anthocyanins may not be similarly absorbed from

the stomach like the non-acylated ones due to steric hindrance, and therefore may act as antioxidants as such in the upper gastrointestinal tract. They possibly reach the intestine intact, and may be absorbed by intestine epithelial cells as seen *in vitro* via glucose transporters. A clinical trial showed that the absorption occurs in a smaller area than that seen for non-acylated anthocyanins. Furthermore, an *in vitro* study conducted with a gastrointestinal model without absorptive cells showed that acylated anthocyanins rapidly degrade in the small intestine, and the unidentified degradants formed in the small and large intestine show radical scavenging activity. Gut microbiota was shown to hydrolyse acylated anthocyanins more readily than the non-acylated ones in rodents, but however, these degradants were not identified. Non-acylated anthocyanins have been shown to be interconverted to other anthocyanidins, further degraded and conjugated, into phenolic metabolites and their conjugates.

Even though various animal studies and clinical trials suggest that anthocyanins and anthocyanin-rich foods affect the postprandial glucose metabolism and inflammation beneficially, the effect of acylation has not been studied thoroughly *in vivo. In vitro* studies show that acylated anthocyanins may alter glycaemia by inhibiting carbohydrate digestion and absorption. Multiple *in vitro* studies show that purple potatoes may decrease the proinflammatory cytokines and enhance the endogenous antioxidative defence. This literature review focused on the acylation of anthocyanins, however, the possible synergistic and antagonistic effects of other flavonoids and other dietary compounds of foods rich in acylated anthocyanins were not considered. Even though the current studies vary highly in different methods, target outcomes, anthocyanin vehicles, anthocyanin purities, anthocyanin structures and administration time-frames, the potential of acylated anthocyanins as bioactive compounds is promising.

As the current published research on acylated anthocyanins has been mainly focused on *in vitro* and animal studies which do not completely represent the complex postprandial metabolism of humans, more clinical trials are required with both normoglycaemic and hyperglycaemic individuals. The structural and quantity aspects of anthocyanins should be taken into account to find the best solutions for food industry and everyday diets. Furthermore, anthocyanins are rarely ingested as such, and thus comparing the effect of the purified anthocyanins and the original anthocyanin-rich food provides insight of the possible matrix effects. More emphasis should be put on a thorough chemical characterisation of the studied foods and extracts often neglected by current literature. Lastly, more studies are needed to fully understand the role of gut microbiota and the anthocyanin degradants and metabolites in the health effects of anthocyanins during the postprandial state.

3 AIMS OF THE STUDY

The aim of this research was to study whether purple-fleshed potatoes (*Solanum tuberosum* L., 'Synkeä Sakari') and their acylated anthocyanins affect postprandial glycaemia, insulinaemia and inflammation, and to examine the phenolic metabolites derived from a meal rich in acylated anthocyanins. The objectives of the three studies were to:

I Investigate if anthocyanin-rich purple potatoes affect postprandial glycaemia and insulinaemia of healthy men in comparison to yellow potatoes

II Assess the effect of purple potato extract rich in acylated anthocyanins on human postprandial glycaemia, insulinaemia and inflammation markers

III Screen the phenolic metabolites and their conjugates excreted into the urine and plasma of healthy study volunteers after an acute intake of purple potato extract rich in acylated anthocyanins

4 MATERIALS AND METHODS

4.1 Clinical interventions

4.1.1 Ethical statements

The Ethics Committee of the Hospital District of Southwest Finland approved the study designs of the clinical interventions **I** and **II**. The first trial was registered at *clinicaltrials.gov* as NCT02005796, and the second as NCT02940080. Volunteers gave their written informed consent before participation. Both clinical interventions were conducted in accordance with the declaration of Helsinki.

4.1.2 Study participants

Fourteen (**Study I**) and seventeen (**Study II**) volunteers from the district of Turku, Finland, participated in the clinical interventions. All participants were healthy non-smoking men, aged between 18 and 45 years. At the screening visit, the body-mass index (BMI 18.5–27 kg/m^2) and blood pressure (<140/80 mmHg) were measured. Next, the study participants participated in a fasting state blood test in the laboratory of the Hospital District of Southwest Finland, and the results were inspected against the reference values stated in **Table 6**. All accepted volunteers had not donated blood or participated in another clinical trial within two months before the first clinical visit.

Table 6. Inclusion criteria for the volunteers monitored with a fasting state blood test.

Target function	Biomarker	Limit of acceptance	
Sugar metabolism	Glucose	4–6	mmol/L
Liver function	Alanine aminotransferase	<60	U/L
Kidney function	Creatinine	<118	µmol/L
Thyroid function	Thyrotropin	0.4–4.5	mU/L
Lipid metabolism	Triglycerides	<2.6	mmol/L
Lipid metabolism	Cholesterol[a]	<5.5	mmol/L
Oxygen transport	Haemoglobin	130–155	g/L

[a]only **Study I**

4.1.3 Study design

Clinical interventions were randomised, single-blinded cross-over trials with one (**Study II**) or two (**Study I**) wash-out periods of two weeks as described in detail in **Figure 7**. The volunteers were asked not to exercise vigorously and to consume only foods and beverages low in flavonoids and fibre (**Table 7**) for 24 h (**I**) or 48 h (**II**) before the clinical visit, and 24 h after consuming the meal (**II**).

After 12 h of fasting, the study participants consumed purple-fleshed potatoes, bilberries or yellow-fleshed potatoes (**I**) or yellow-fleshed potatoes with and without purple potato extract rich in acylated anthocyanins (PPE) (**II**), described in detail in the next chapter. No food or drink was allowed for 4 h after the meal. Venous blood samples were drawn at the fasting state, and 20, 40, 60, 90, 120, 180 and 240 min postprandially for both clinical trials. For the second clinical trial, urine samples were also collected at fasting state, and 0–4, 4–8, 8–12 and 12–24 hours after the meal.

The number of study participants in **Study I** was decided on the basis of the previously published clinical postprandial interventions conducted by our research group[197,311] which involved ten participants. The required sample size for **Study II** was deduced with power calculations based on **Study I**, resulting in 15 participants. Statistical power and effect size were calculated for significant effect of added PPE extract (smaller postprandial plasma glucose in comparison to yellow potato meal; *t*-test, $p < 0.05$) using the G*power software.[312,313] The actual number of participants was higher than these calculations required as 13 and 17 volunteers participated in **Studies I** and **II**, respectively.

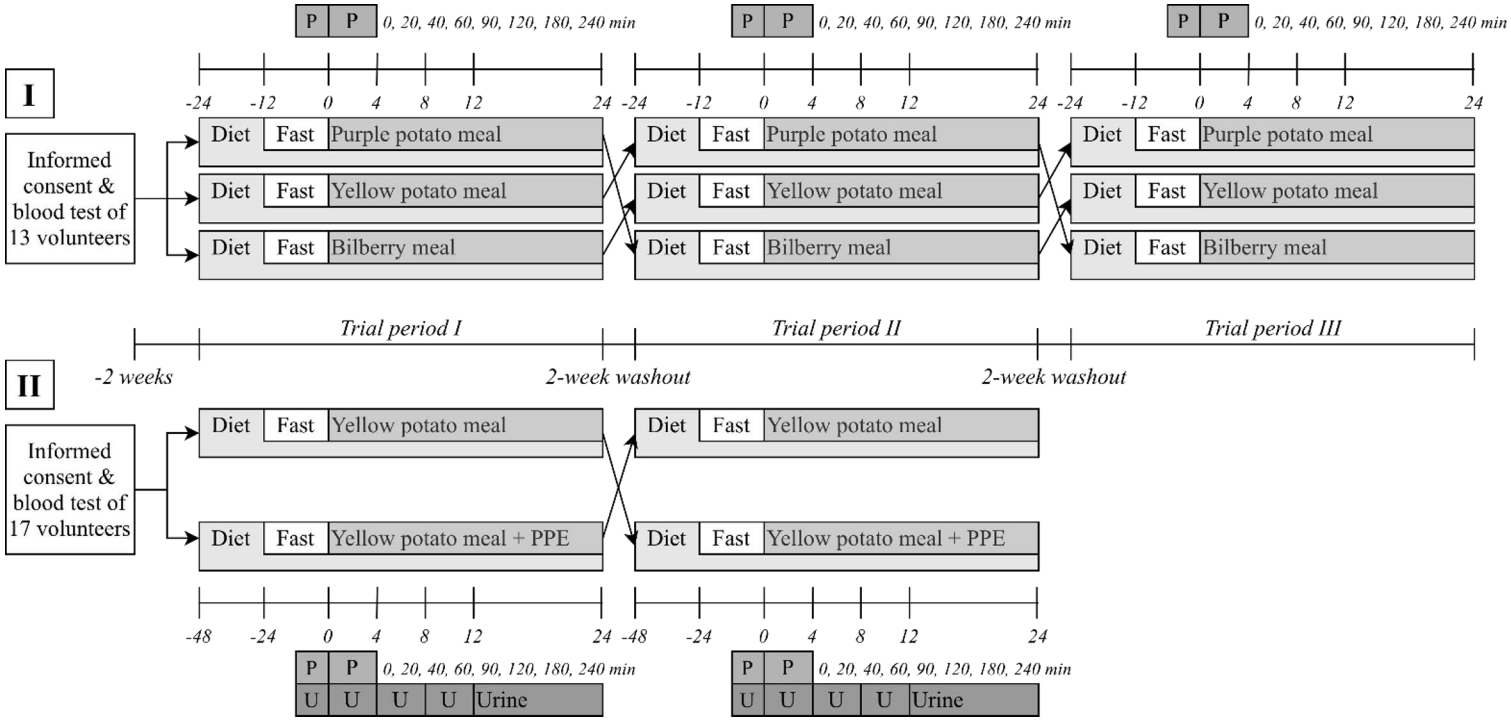


Figure 7. Overview of the postprandial clinical trials **I** and **II**. Consumption of the test meal is considered as the time point 0. Plasma was sampled, but urine was collected as total voids. Diet refers to a study diet low in flavonoids and fibre. P, plasma; U, urine; PPE, purple potato extract.

Table 7. Study diet low in flavonoids and fibre (**Studies I** and **II**).

Food type	Allowed	Avoidable
Animal-based products and their substitutes		
Dairy products	Milk, sour milk, natural and vanillin yoghurt, quark, cream, cheese, vanillin ice cream	Flavoured products with other than vanillin flavour
Eggs	All	None
Meat, poultry, seafood	All (no marinade)	Breaded and/or processed products such as nuggets, sausages and meatballs
Meat substitutes	None	All seeds, beans, nuts, tofu
Berries, fruits, root vegetables and vegetables		
Berries and fruits	One daily banana	All others
Vegetables, root vegetables	None	All, *incl.* potatoes, onions and tomato sauce
Beverages		
Alcohol	None	All
Refreshments	None	Coffee, tea, chocolate drinks, juices, soft drinks, energy drinks
Water	Non-flavoured Still and sparkling	
Grains and grain products		
Accompaniment	White pasta and white rice	All others, *incl*. whole grain products and noodles
Bakery products	White bread	All other breads, cakes, cookies, cupcakes. Whole grain products, seed-containing products
Breakfast grains	White bread	Porridge, breakfast cereals, muesli
Desserts	Sweet bun without cinnamon and cardamom	All, including chocolate
Oils and fats		
	Butter	Oils, margarines, other spreads
Spices and sweeteners		
Spices	Salt and black pepper	Other spices, herbs, sauces and marinades
Sweeteners	Sugar	Other sweeteners, honey
Supplements		
Dietary supplements	None	All, *e.g.* vitamins, minerals, extracts and concentrates

4.1.4 Study meals

4.1.4.1 Origins of the study foods

The origins of the foods used for the meals are listed in **Table 8**. All foods were commercially purchased except for the purple potatoes, which were kindly provided by the farmer, PhD Mikko Griinari. Synkeä Sakari is a *Solanum tuberosum* L. ′Hankkijan Timo′ purple landrace cross. The used potato cultivars are classified as floury.

Table 8. The foods used in the preparation of the test meals.

	Food	Variety	Origin	Location in Finland
I	Purple potatoes	*Solanum tuberosum* L. ′Synkeä Sakari′	PhD Mikko Griinari	Maalahti & Kotka
I	Yellow potatoes	*Solanum tuberosum* L. ′Van Gogh′	Lapinojan ruokaperuna Oy	Kalajoki
I	Bilberries	*Vaccinium myrtillus* L.	Kiantama Oy	Suomussalmi
II	Purple potatoes	*Solanum tuberosum* L. ′Synkeä Sakari′	PhD Mikko Griinari	Kokemäki & Muhos
II	Yellow potatoes	*Solanum tuberosum* L. ′Afra′	Veljekset Kitola Oy	Nousiainen

4.1.4.2 Extraction of the purple potato anthocyanins

The acylated anthocyanins of purple potatoes were extracted with an optimised method as earlier described[314] for the intervention in **Study II**. Briefly, 19 kg of purple potatoes were freeze-dried and extracted twice using 20 vol-% aqueous ethanol containing seven vol-% acetic acid for six hours. The extracts were purified with Amberlite XAD-16 adsorbent in a glass column and eluted with 75 vol-% ethanol containing seven vol-% of acetic acid. The extraction yield was 1.2 L of anthocyanin-rich purple potato extract (PPE) with a dark purple colour.

4.1.4.3 Preparation of the study meals

The unpeeled potatoes for **Studies I** and **II** were steam-cooked in water (0.7 mL/g potatoes). The cooked potatoes were mashed with all the remaining cooking water. The potatoes were divided into portions containing 350 g of the steam-cooked potatoes, with an addition of 106 g (yellow potato meal) or 109 g (purple potato meal) of cooking water for **Study I**, and 111 g for **Study II**. The portions were stored at –18 ºC until the clinical trials.

In **Study I**, the potato mashes were thawed in a refrigerator over night, and then gently heated in a microwave oven (160 W) until warm. The bilberries were served in gelatinised potato starch freshly prepared by heating 65.5 g potato starch (Finnamyl Oy, Kokemäki, Finland) and 342 mL water and adding 51 g of frozen bilberries. All meals were served with a glass of water (250 mL).

In **Study II**, the meals consisted of yellow-fleshed potato portions with a meal additive of 30 mL of PPE (corresponding to an extract from 0.48 kg of fresh purple potatoes) for the study meals, and 30 mL of water for the control meals. As PPE contained acetic acid[314], the pH of the meal additives was adjusted to 4 by adding 9.1 mmol of acetic acid in the form of synthetic vinegar (Maustaja, Pyhäntä, Finland) to the control meal additive, and by adding 9.5 mmol and 1.7 mmol of food-grade sodium hydroxide (J.T.Baker, Deventer, Holland) to the study meal additive and the control meal additive, respectively. The amount of sodium was standardised between the meals by adding 0.4 g of sodium chloride into the control meal additive. The total volume of the meal additives was set to 40 mL per meal and stored at –18 °C. The potato portion was thawed overnight in a refrigerator, heated in the morning, allowed to cool down to room temperature and then either the study or control meal additive was added with 10 mL of additional rinsing water and mixed carefully. The meals were served with a glass of water (300 mL).

4.1.4.4 Justification of the portion sizes

The portion sizes used in **Study I** were decided as a compromise between the previously used[201] bioactive doses of anthocyanins, an adequate amount of energy for the volunteers and the pleasantness of the served meal size. The bilberry serving size was decided by standardising the amount of anthocyanins in the meal so as to be the same as in the purple potato portion analysed with HPLC. The amount of digestible carbohydrates in the purple potato meal was standardised by adding the corresponding amount of potato starch to the bilberry meal. For **Study II**, the amount of potato mash and the added anthocyanin extract was decided based on **Study I**.

4.2 Chemical characterisation of the meals

4.2.1 Nutrient content

The nutrient content of the study and control meals were analysed as served (**Study I**) or without the meal additives (**Study II**) using standardised methods. The moisture content was determined with NMKL 23:1991; the fat content, AOAC 922.06 2002 and FIL-IDF 5B:1986; the protein content, the Kjeldahl

method (NMKL 6:2003); the fibre content, AOAC 985.29 2003 (only **Study I**) and the ash content, NMKL 173:2005. The total amount of digestible carbohydrates was determined by subtracting the amounts of moisture, protein, fat, fibre and ash from the fresh weight, and the energy content was calculated based on the amounts of carbohydrates, proteins, fat and fibre in the portions. The starch content of the yellow potato meal (**II**) was analysed using spectrophotometric methods.

4.2.2 Acetic acid and ethanol

Acetic acid and ethanol concentrations were investigated as the PPE was extracted with acetic acid and ethanol.[314] The analyses were performed with gas chromatography (GC) equipped with a flame-ionization detector (FID) from Hewlett-Packard 5890 Series II gas chromatograph (Hewlett-Packard Co, Palo Alto, CA). The injection volume was 0.2 µL, and helium was used as a carrier gas with a total flow rate of 118.0 mL/min in split mode, of which 3.7 mL/min was directed to the column. The volatile compounds were separated with an EC-WAX (30 m × 0.53 mm, 1.2 µm, Alltech, Nicholasville, KY) column. The temperature of the column oven was first set to 80 °C, held for 5 minutes, then increased by 10 °C/min until 240 °C and held for 10 minutes. Acetic acid and ethanol concentrations were determined using external standard curves.

4.2.3 Free sugars and organic acids

Freeze-dried bilberries (1 g, **Study I**), mashed purple potatoes and yellow potatoes (2 g, **Study I**) and yellow potato portion (2 g, **Study II**) were extracted three times with MQ water. The samples were analysed as volatile tetramethylsilane (TMS) derivatives with the method described previously[315,316] using two GC systems from Hewlett-Packard Co (Palo Alto, CA) (**I**) and Shimadzu (Kioto, Japan) (**II**), both equipped with an FID. An aliquot of 0.2 µL was injected and the derivatives were separated with a non-polar Simplicity-1 fused silica capillary column (30 m × 0.25 mm, i.d. 0.25 µm, Supelco, Bellefonte, PA) (**I**) or the non-polar poly(dimethyl siloxane) SPB-1 (30 m × 0.25 mm, i.d. 0.25 µm, Supelco, Bellefonte, PA) (**II**). The TMS derivatives were identified with the corresponding standard compounds and quantified using internal standards sorbitol and tartaric acid using compound-specific correction factors.

4.2.4 Anthocyanins

The anthocyanins of the potato meals and bilberries (**I**) and the yellow potato meal (**II**) were extracted, or diluted in the case of PPE (**II**), with methanol and hydrochloric acid (99:1, v/v) using the modified method described earlier.[316,317] Anthocyanins were quantified as cyanidin-3-*O*-glucoside equivalents with the external standard method. In **Study I**, the total anthocyanin content was determined using a spectrophotometer at λ_{max} 525 nm. In **Studies I** and **II**, reversed-phase high-performance liquid chromatography (HPLC) (Shimadzu, Kyoto, Japan) with a diode array detector (DAD) at 190–600 nm was used to separate and quantify the anthocyanins. In **Study I**, a Kinetex C18 column (2.6 µm, 100 × 4.60 mm, Phenomenex, Torrance, CA) was used at 35 °C using a modified gradient program[318] whereas in **Study II**, the chromatographic separation of the purple potato anthocyanins was optimised using a Kinetex Polar C18 column (2.6 µm, 150 × 4.60 mm, Phenomenex, Torrance, CA). The injection volume was 10 µL. The anthocyanins were monitored at λ_{max} 520 nm.

The anthocyanins were identified with a UHPLC–DAD–ESI–MS/MS (Waters Corp., Milford, MA) (**I**, **II**) in a positive ion mode by obtaining full scan mass spectra at a mass range of *m/z* 100–1,400. The product ions were followed after colliding the selected precursor ions in the second quadrupole. The exact masses of anthocyanins were determined in **Study II** using a high-resolution UHPLC–DAD–ESI–Q-ToF–MS/MS (Bruker Daltonik GmbH, Bremen, Germany) in a positive autoMS/MS mode. The wavelength range of 190–800 nm was scanned with DAD, and the mass range of *m/z* 20 to 1,000 was recorded. The literature using nuclear magnetic resonance spectroscopy (NMR) to characterise the potato anthocyanins were used in the identification.[65,70]

4.2.5 Flavonols, flavonol glycosides and hydroxycinnamic acids

Flavonols, flavonol glycosides and hydroxycinnamic acid derivatives were extracted three times with ethyl acetate[317,319] from the potato meals and bilberries (**I**) and the yellow potato meal and the PPE (**II**). Flavonols, flavonol glycosides and hydroxycinnamic acids were identified (**I, II**) and quantified (**II**) with an HPLC–DAD (Shimadzu, Kyoto, Japan)[320] scanning at the wavelength range of 190–600 nm. Ten µL of the samples was injected, and the analytes were separated using an Aeris peptide XB-C18 (3.6 µm, 150 × 4.60 mm, Phenomenex, Torrance, CA) column at 30 °C. In **Study II**, quantification was conducted at λ_{max} 320 nm for the hydroxycinnamic acid and caffeoylquinic acid derivatives, and at λ_{max} 354 nm for the flavonol glycosides using the external standard method. The caffeoylquinic acids were calculated as the equivalents of 3-

caffeoylquinic acid, other hydroxycinnamic acid derivatives as caffeic acid equivalents and flavonol glycosides as quercetin-3-*O*-rutinoside equivalents.

Identification of flavonols, flavonol glycosides and hydroxycinnamic acid derivatives was conducted with a UHPLC–DAD–ESI–MS/MS operating in both negative and positive ion modes. The full scan mass ranged from *m/z* 130 to 700 (**I**) or 800 (**II**). In **Study II**, the exact masses were measured using the high-resolution UHPLC–DAD–ESI–Q-ToF–MS/MS (Bruker Daltonik GmbH, Bremen, Germany) in negative ion and autoMS/MS modes at *m/z* 20–1,000. External standards were used for identification when available.

4.2.6 Total phenolic compounds

The total number of phenolic compounds (**I**) was determined using a spectrophotometric method utilising the reactions of a Folin–Ciocalteu reagent and Na_2CO_3 with the analytes[321]. Quantification was performed at λ_{max} 765 nm with the external standard method using gallic acid.

4.3 Biomarkers of postprandial state

4.3.1 Glucose and insulin

Plasma glucose concentration was quantified with an enzymatic method (hexokinase) and the insulin with an electrochemiluminescence immunoassay (ECLIA) for **Studies I** and **II**. Both analyses were conducted with a Cobas 8000 analyser utilising the c702 module and the CobasVR GLUC3, Glucose HK Gen.3 kit in the glucose analyses and e602 module and the CobasVR Insulin, Elecsys kit in the insulin analyses (Roche Diagnostics, Rotkreuz, Switzerland). The incremental areas under the glucose and insulin concentration curves (iAUC) after each meal were calculated until the glucose and insulin levels reached the fasting level in accordance with the trapezoidal rule.

4.3.2 Inflammation markers

In **Study II**, 92 inflammation markers, such as various interleukins, CC chemokines, CXC chemokines, monocyte chemotactic proteins and matrix metalloproteinases were measured from the plasma samples collected at the fasting state and 240 min after the meals using a cDNA-based multiplex immunoassay and qPCR (Olink Proteomics, Uppsala, Sweden).

4.3.3 Urinary creatinine

For normalisation, creatinine was analysed from the urine samples (fasting state, 0–4, 4–8, 8–12 and 12–24 h) collected in **Study II** with enzymatic methods utilising a Cobas C702 automatic analyser (Roche Diagnostics GmbH, Mannheim, Germany).

4.4 Phenolic metabolites

In **Study III**, the urine and plasma samples from **Study II** were purified with a solid-phase extraction method modified from a previous method[183] using the StrataX cartridges (6mL, 500 mg) from Phenomenex (Torrance, CA) with 0.25% formic acid in methanol as the elution solvent. The extracted samples were concentrated and further reconstituted with 0.1% formic acid. Internal standard caffeine-d_9 (200 mM) was used to control the volume of the evaporated samples. The samples were divided and those used for the anthocyanin analyses were acidified with formic acid.

The targeted anthocyanins and phenolic metabolites were analysed from the pre-processed samples with two separate and extensive multiple reaction monitoring (MRM) panels of 123 (anthocyanins) and 113 (other phenolics) compounds with a UHPLC–ESI–MS/MS (Agilent Technologies, Santa Clara, CA). The targeted MRM panels contained both predicted metabolites and those presented in the current literature for non-acylated anthocyanins as the literature on the metabolites of acylated anthocyanins is limited. Accordingly, a large library of phenolic compounds (**Table 9**), of which many were synthesised for this study, were used in the identification and quantification of the analytes.

Five µL of a solid-phase extracted urine sample or one µL of a plasma sample were injected into an Agilent Technologies 1290 Infinity UHPLC, and the analytes were separated with ACQUITY UPLC HSS T3 1.8 µm (2.1 × 100 mm, Waters, Milford, MA) column at 35 ºC. Quantification of the compounds identified with a corresponding standard compound was performed with the external standard method. Tentatively identified compounds were not quantified but the analytical response areas were presented as a heatmap to compare their postprandial trends. Additionally, the pharmacokinetic iAUC values were calculated using the trapezoidal rule, and C_{max} and t_{max} were determined as the mean of the maximal values of the individual volunteers. For urine, t_{max} was determined on the basis of the most frequent categorical time period variable.

Table 9. Standard compounds available in **Study III**.

Compound	Origin
Caffeic acid	Sigma-Aldrich (St Louis, MO)
Caffeine-(trimethyl-d_9)	Sigma-Aldrich (St Louis, MO)
Catechol	Sigma-Aldrich (St Louis, MO)
trans-Cinnamic acid	Sigma-Aldrich (St Louis, MO)
Chlorogenic acid	Sigma-Aldrich (St Louis, MO)
p-Coumaric acid	Sigma-Aldrich (St Louis, MO)
Cyanidin-3-*O*-glucoside	Extrasynthese (Genay, France)
Cyanidin-3-*O*-rutinoside	Extrasynthese (Genay, France)
Delphinidin-3-*O*-glucoside	Extrasynthese (Genay, France)
Delphinidin-3-*O*-rutinoside	Extrasynthese (Genay, France)
Ferulic acid	Sigma-Aldrich (St Louis, MO)
Gallic acid	Sigma-Aldrich (St Louis, MO)
Gallic acid-3- and -4-*O*-glucuronides	Dr. P. Needs (QIB, Norwich, UK)
Hippuric acid	Sigma-Aldrich (St Louis, MO)
Homoprotocatechuic acid	Sigma-Aldrich (St Louis, MO)
Homovanillic acid	Sigma-Aldrich (St Louis, MO)
4-Hydroxybenzaldehyde	Sigma-Aldrich (St Louis, MO)
3- & 4-Hydroxybenzoic acids	Sigma-Aldrich (St Louis, MO)
4-Hydroxybenzyl alcohol	Sigma-Aldrich (St Louis, MO)
5-Hydroxyferulic acid	Sigma-Aldrich (St Louis, MO)
alpha-Hydroxyhippuric acid	Sigma-Aldrich (St Louis, MO)
4-Hydroxyphenylacetic acid	Sigma-Aldrich (St Louis, MO)
Isoferulic acid-3-*O*-glucuronide	Dr. P. Needs (QIB, Norwich, UK)
Isovanillic acid	Sigma-Aldrich (St Louis, MO)
Malvidin-3-*O*-glucoside	Extrasynthese (Genay, France)
2-Methoxybenzoic acid	Sigma-Aldrich (St Louis, MO)
3-*O*-Methyl-gallate	Sigma-Aldrich (St Louis, MO)
3- & 4-Methyl hippuric acids	Sigma-Aldrich (St Louis, MO)
Methyl-3,4-dihydroxybenzoate	Sigma-Aldrich (St Louis, MO)
Methyl vanillate	Sigma-Aldrich (St Louis, MO)
Pelargonidin-3-*O*-glucoside	Extrasynthese (Genay, France)
Pelargonidin-3-*O*-rutinoside	Extrasynthese (Genay, France)
Phloroglucinol	Sigma-Aldrich (St Louis, MO)
Phloroglucinaldehyde	Sigma-Aldrich (St Louis, MO)
Protocatechuic acid	Sigma-Aldrich (St Louis, MO)
Protocatechuic acid-3- and -4-*O*-glucuronides	Dr. P. Needs (QIB, Norwich, UK)
Protocatechuic acid-3- and -4-*O*-sulfates	Dr. P. Needs (QIB, Norwich, UK)
Protocatechuic aldehyde	Sigma-Aldrich (St Louis, MO)
Pyrogallol	Sigma-Aldrich (St Louis, MO)
Quercetin	Sigma-Aldrich (St Louis, MO)
Quercetin-3-*O*-rutinoside	Sigma-Aldrich (St Louis, MO)
Sinapic acid	Sigma-Aldrich (St Louis, MO)
Syringic acid	Sigma-Aldrich (St Louis, MO)
Taxifolin	Sigma-Aldrich (St Louis, MO)
Vanillic acid	Sigma-Aldrich (St Louis, MO)

4.5 Statistical analyses

The normality of the probability distribution of the data was tested in each study with the Shapiro–Wilk test at the significance level of 0.05. An ANOVA (analysis of variance) for repeated measurements was used for the multiple comparisons performed in **Study I** with Bonferroni corrections. In both **Studies I** and **II**, depending on the normality of the data, a paired sample *t*-test or the Wilcoxon signed rank test was used to test the differences between the meal-dependent variables using the significance level of 0.05. Statistical analyses for the glucose and insulin concentrations (**I, II**) were performed using IBM SPSS Statistics 23.0 software (SPSS Inc, Chicago, IL). Statistical analyses for the inflammation marker data (**II**) and **Study III** were conducted with R 3.5.1[322] with the Effsize package 0.7.4[323].

In **Study II**, the effect size measures of Cohen's *d* and *r* score were determined for the parametric and non-parametric tests, respectively. The *r* score was calculated using the equation $r = Z / \sqrt{N}$, in which Z is the test measure of the Wilcoxon signed rank test and *N* is the total number of observations. The data was interpreted using the following reference criteria: ≤ 0.2, small effect size; ≤ 0.5, medium effect size, and ≤ 0.8, large effect size. The adjusted *p*-values (here, the *q*-values) were calculated using the Benjamini–Hochberg method.

In **Study III**, the tentatively identified data set was heatmapped using R 3.5.1[322] with the packages gplots version 3.0.1.1[324] and RColorBrewer version 1.1.2[325] to show a general overview of the postprandial behavior of the compounds. The difference between the volume corrected metabolite areas between the study and control meals were calculated and resulted in a variable describing change between the meals at certain time points or periods (delta). As the levels of different metabolites varied considerably, the fasting state value of the delta variable was set to zero by subtracting it from all time points within the compound in question. Then, the values were normalised to a range [-1, 1] by dividing with the absolute maximum delta of the corresponding compound.

5 RESULTS AND DISCUSSION

5.1 Characterisation of the test meals

The test meals served in the clinical trials **I** and **II** were characterised carefully for the contents of nutrients, sugars, organic fruit acids, acetic acid, anthocyanins and other phenolic compounds (**Table 10**). The potato meals were similar in nutrient composition, and they provided 70.5, 68.9 and 75.1 g / meal of available carbohydrates in the purple potato meal (**Study I)**, yellow potato meal (**Study I**) and yellow potato portion (**Study II**), respectively. The main free sugar in the yellow potato meals was glucose (0.7 g, **Study I**; 1.4 g, **Study II**), followed by fructose (0.6 g, **Study I**; 0.8 g, **Study II**). In the purple potato meal used in **Study I**, the main sugar was sucrose (0.8 g). The purple potato extract contained only low amounts of free sugars as extraction residues. The study meals of the clinical interventions contained comparable amounts of anthocyanins (**Study I**, 138.8 mg; **Study II**, 152.4 mg). The yellow potato mash used as a control meal (**Study II**) contained 0.7 mg flavonol glycosides and 4.5 mg hydroxycinnamic acid derivatives as such, and the PPE contained an additional 140.1 mg of hydroxycinnamic acid derivatives. Comparing to other clinical trials feeding anthocyanin-rich foods, the doses of anthocyanins typically range from 100 mg up to 700 mg of anthocyanins[196,198,199,201,202] and thus, the anthocyanin doses in the study meals of **Study I** and **II** were on the lower end.

Table 10. Contents of nutrients, organic acids, ethanol, acetic acid and phenolic compounds per meal given as mean ± standard deviation. The purple potato extract (30 mL) was added to yellow potato portion to form the study meal (**II**). Modified from **Studies I** and **II**.

Content per meal	Purple potato meal (**I**)	Yellow potato meal (**I**)	Bilberry meal (**I**)	Yellow potato portion (**II**)	Purple potato extract (**II**)
Nutrients					
Energy (kJ)	1390 ± 33	1318 ± 3.2	1142 ± 39	1364 ± 26	NA
Fat (g)	trace	trace	trace	trace	NA
Digestible carbohydrates (g)	70.5 ± 1.6	68.9 ± 0.0	67.2 ± 2.3	75.1 ± 1.3	NA
Starch (g)	NA	NA	NA	66.8 ± 3.3	NA
Protein (g)	8.0 ± 0.3	5.0 ± 0.0	0.5 ± 0.0	4.6 ± 0.0	NA
Fibre	7.0 ± 0.4	6.1 ± 0.5	trace	NA	NA
Moisture (g)	80.6 ± 0.4	81.7 ± 0.1	85.2 ± 0.4	81.9 ± 0.3	NA
Ash (g)	3.7 ± 0.0	3.2 ± 0.0	trace	3.5 ± 0.3	NA
Ethanol (mmol)	NA	NA	NA	NA	0.8 ± 0.0
Free sugars					
Fructose (mg)	68.1 ± 3.4	564.7 ± 19.3	1996.0 ± 333.1	848.6 ± 17.3	0.8 ± 0.1
Glucose (mg)	76.1 ± 3.8	675.9 ± 22.9	2158.5 ± 212.0	1398.2 ± 63.7	4.4 ± 0.5
Sucrose (mg)	807.7 ± 43.5	423.9 ± 24.8	0.1 ± 0.0	646.7 ± 26.0	0.4 ± 0.0
Fruit acids and organic acids					
Acetic acid (mmol)	NA	NA	NA	NA	54.9 ± 0.7
Ascorbic acid (mg)	21.5 ± 2.4	19.5 ± 3.2	83.2 ± 2.5	ND	ND
Citric acid (mg)	1029.5 ± 79.1	816.9 ± 43.5	256.6 ± 49.0	904.6 ± 34.9	9.1 ± 0.2
Malic acid (mg)	170.0 ± 7.0	224.2 ± 5.5	103.1 ± 7.2	283.2 ± 10.0	1.1 ± 0.0
Quinic acid (mg)	17.2 ± 0.1	35.1 ± 0.9	295.3 ± 62.8	37.2 ± 1.5	0.3 ± 0.1
Phenolics					
Anthocyanins (mg)	138.8 ± 6.5	ND	215.2 ± 6.3	ND	152.4 ± 3.8
Flavonol glycosides (mg)	NA	NA	NA	0.7 ± 0.1	ND
Hydroxycinnamic acids (mg)	NA	NA	NA	4.5 ± 0.5	140.1 ± 0.5

NA, not analysed; ND, not detected

5.2 Identification of acylated anthocyanins and hydroxycinnamic acids

The composition of acylated anthocyanins in the studied purple potatoes were preliminary identified in **Study I**, but for **Study II**, the chromatographic separation was further optimised to enhance the separation, and subsequently the identification, of anthocyanins. The anthocyanin pattern of the PPE was complex; 18 peaks were detected of which three contained more than one acylated anthocyanin (**Figure 8**). The anthocyanins were identified as acylated derivatives (99%) of petunidin, peonidin, cyanidin, malvidin, delphinidin and pelargonidin, of which petunidin and peonidin were the most abundant ones. Acylated anthocyanins had distinctive fragmentation patterns; the loss of 162 amu was a glucose, and 454 amu, 470 amu and 484 amu were the losses of a rutinose linked to an acyl group (coumaric acid, caffeic acid and ferulic acid, respectively). The major anthocyanin was a petunidin-coumaroyl-rutinoside-glucoside (60% of the total anthocyanin content) followed by peonidin-coumaroyl-rutinoside-glucoside (16%).

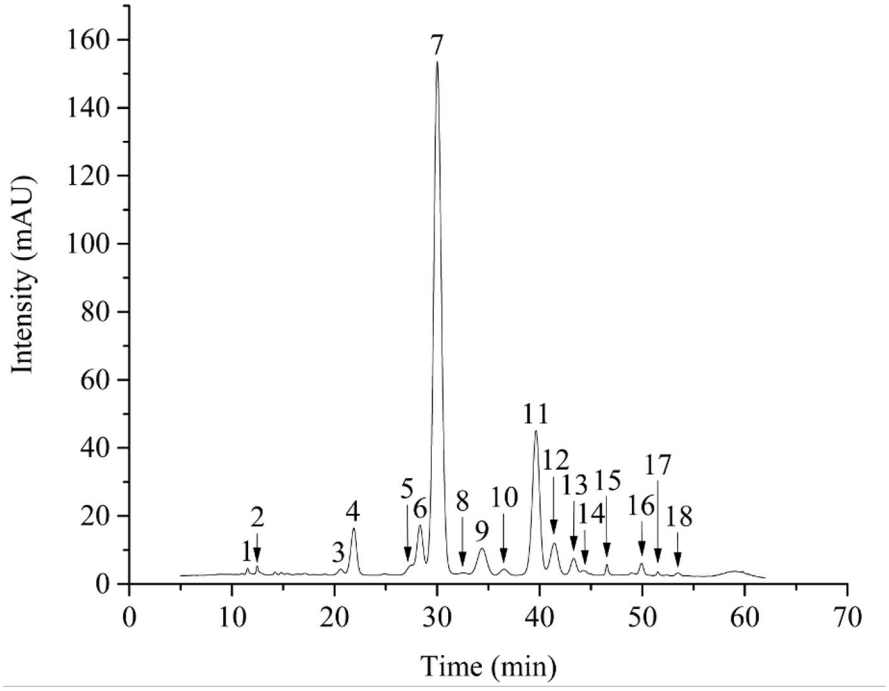


Figure 8. The DAD chromatogram at 520 nm of the purple potato extract (PPE) rich in acylated anthocyanins. The peak numbering refers to **Table 11**. Reprinted from publication **II**[326] with the permission of Elsevier.

Table 11. Anthocyanin composition in the purple potato extract (**II**). Numbering refers to **Figure 8.**

	Anthocyanin		Anthocyanin
1	Petunidin-rutinoside-glucoside	**10**	Pelargonidin-coumaroyl-rutinoside-glucoside
2	Peonidin-rutinoside-glucoside	**11**	Peonidin-coumaroyl-rutinoside-glucoside
3	Petunidin-coumaroyl-rutinoside-glucoside; cyanidin-caffeoyl-rutinoside-glucoside	**12**	Malvidin-coumaroyl-rutinoside-glucoside
		13	Peonidin-feruloyl-rutinoside-glucoside
4	Petunidin-caffeoyl-rutinoside-glucoside; delphinidin-coumaroyl-rutinoside-glucoside	**14**	Malvidin-feruloyl-rutinoside-glucoside
		15	Petunidin-coumaroyl-rutinoside
5	Peonidin-caffeoyl-rutinoside-glucoside	**16**	Petunidin-coumaroyl-rutinoside-acetyl-glucoside
6	Cyanidin-coumaroyl-rutinoside-glucoside; peonidin-coumaroyl-rutinoside-glucoside	**17**	Peonidin-coumaroyl-rutinoside-acetyl-glucoside
7	Petunidin-coumaroyl-rutinoside-glucoside		
8	Unknown 1	**18**	Unknown 2
9	Petunidin-feruloyl-rutinoside-glucoside		

Caffeic acid and 3-caffeoylquinic acid were identified in the purple and yellow potatoes (**I**) and the PPE (**II**), whereas a minor amount of 4-caffeoylquinic acid was found in the PPE and the yellow potato meal (**II**). The yellow potato meal also contained a trace amount of 5-caffeoylquinic acid (**II**). In **Study II**, some minor derivatives were found, such as coumaroyl-rhamnoside hexosides at *m/z* 473 and a caffeoyl-rhamnoside derivative at *m/z* 515 identified on the basis of their fragmentation patterns. The structures of the main hydroxycinnamic acid derivatives of potatoes are shown in **Figure 9**.

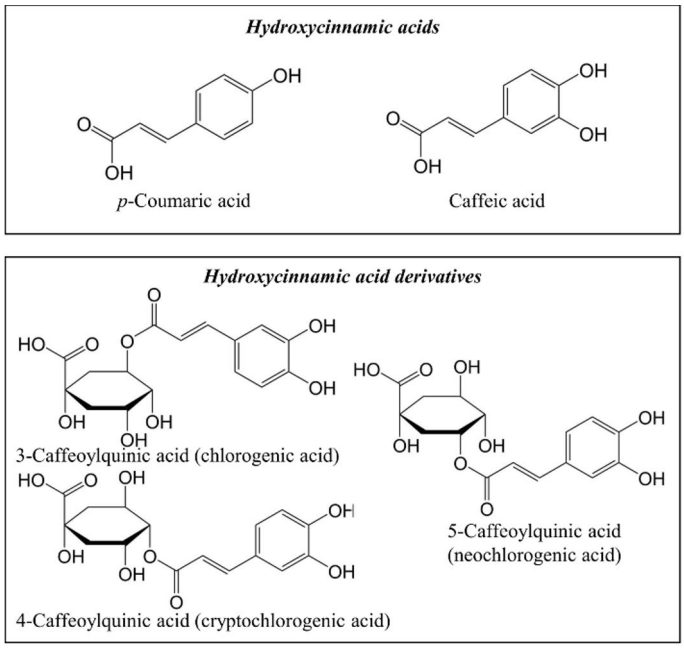


Figure 9. The hydroxycinnamic acid derivatives found in potatoes (**I**, **II**).

The anthocyanin content of Synkeä Sakari (48 mg/100 g FW) is slightly higher than the average content of other European pigmented potato varieties (0.7–74 mg/100 g FW)[45], average being 41.3 mg/100 g FW[44]. The anthocyanin profile of Synkeä Sakari was typical for a purple potatoes as petunidin and peonidin are the most common anthocyanidins in purple varieties in general[41,65–67,70]. However, detecting all six anthocyanidins was surprising. From the non-anthocyanin phenolic compounds, chlorogenic acid (3-caffeylquinic acid) is the most common one in potatoes. The average concentration of chlorogenic acid in potatoes is 269 mg/100 g FW followed by caffeic acid, 67.5 mg/ 100 g FW.[44] Other common minor phenolic compounds detected in pigmented potatoes are 4- and 5-caffeoylquinic acids, other caffeic acid derivatives, ferulic acid, *p*-coumaric acid, quercetin-3-*O*-glucoside and kaempferol-3-*O*-rutinoside.[57,68,72,75]

5.3 Postprandial glucose and insulin

In **Study I**, the purple potato meal decreased the postprandial plasma glucose and insulin in comparison to the yellow potato meal (**Figure 10 A, B**). The difference in plasma glucose was statistically significant at 40 min ($p = 0.044$) after the meal. The total insulinaemia was decreased when calculated as the incremental area of the insulin curve until 120 minutes after the meal ($p = 0.012$). The purple potato meal also caused lower blood glucose when compared to the bilberry meal at 20 min ($p = 0.001$) and 40 min ($p = 0.032$), and at iAUC120 and 240 min ($p = 0.007$ and $p = 0.017$, respectively). The purple potato meal also caused lower postprandial insulin at 20 min ($p = 0.045$) and iAUC at 120 min ($p = 0.012$) as compared to the bilberry meal. The plasma glucose was decreased after the bilberry meal compared to the yellow potato meal at 20 min ($p = 0.025$), and iAUC at 120 min and 240 min for insulin were decreased ($p = 0.012$ and $p = 0.007$).

In **Study II** (**Figure 10 C, D**), the anthocyanin-rich purple potato extract decreased the postprandial glucose statistically significantly at 20 min and 40 min compared with the control meal ($p = 0.015$ and 0.004, respectively), but at 240 min, the blood glucose level was elevated compared to the control ($p = 0.023$). The PPE lowered the incremental area under the glucose curve calculated until 120 minutes ($p = 0.019$). Furthermore, the PPE caused lower plasma insulin responses at 20, 40 and 60 minutes after the meal ($p = 0.003$, 0.004, 0.005, respectively), and increased it at 180 and 240 minutes compared to the control meal ($p = 0.004$ and 0.006, respectively). The incremental area under the insulin curve calculated until 120 minutes was decreased ($p = 0.015$) after the study meal compared to the control meal.

Therefore, our results suggest that an acute intake of purple potatoes decrease postprandial glycaemia and insulinaemia as compared to yellow potatoes in healthy men. The acylated anthocyanins may contribute to these effects as the anthocyanin-rich purple potato extract had postprandial hypoglycaemic and hypoinsulinaemic effects by suppressing the steep rise of blood glucose and insulin after anthocyanin-rich meal at 20–60 minutes. The PPE also prevented the glucose and insulin levels from decreasing below the fasting state level which was observed after the control meal.

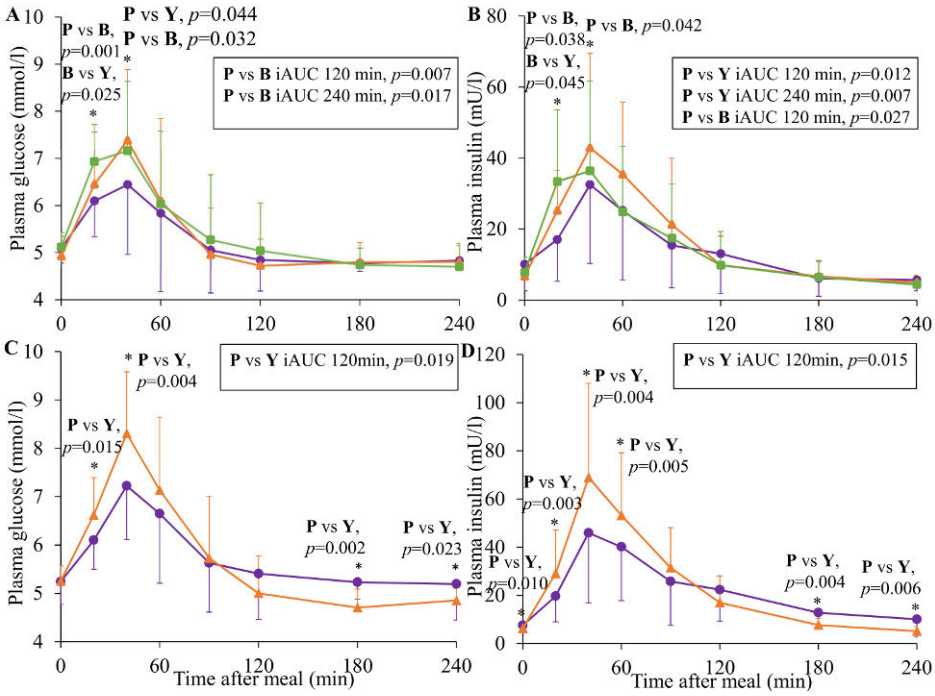


Figure 10. Plasma glucose (A, *n*=13; C, *n*=17) and insulin (B, *n*=12; D, *n*=17) measured in **Studies I** (A, B) and **II** (C, D). **P**, purple potato meal (**I**) or the PPE (**II**) (●); **Y**, yellow potato meal (▲); **B**, bilberry meal (■). Values are given as mean ± standard deviation. Adapted from the publications **I**[327] and **II**[326] with the permission of Taylor & Francis and Elsevier, respectively.

Previous literature shows that purple potato chips (50 g of available carbohydrates) have a statistically insignificant trend of decreasing the postprandial glycaemic response (AUC) in healthy volunteers when compared to white and red potato chips, and that the red and purple potato chips delay the postprandial peak time of glucose when compared to white potato chips (Chapter

2.3, Table 4).[215] A comparison of a meal of oven-baked purple, red and white potatoes with a dose standardised to 50 g of available carbohydrates (290–380 g of potato) did not cause statistically significant difference in the glycaemic or insulinaemic responses. The amount of anthocyanins per portion was, however, low (15 and 16 mg/100 DW for the red and purple varieties, respectively).[211] A long-term study conducted with hypertensive and overweight/obese volunteers showed that the intake of purple potatoes twice per day for four weeks did not decrease the fasting state glycaemic response.[213] In long-term rat studies, a purple potato extract caused a statistically insignificant decrease of fasting state glucose in diabetic rats[216] and a lyophilised purple potato powder caused a significant decrease in the third week[214] in comparison to the baseline diet. Another long-term study conducted with lyophilised purple potatoes decreased blood glucose in obese rats in comparison to white potatoes after glucose and insulin tolerance tests.[212] Oral administration of diacylated anthocyanins from purple sweet potatoes decreased the postprandial glycaemic response of normoglycaemic rats[194] and hyperglycaemic mice[195]. Several postprandial studies conducted with foods rich in non-acylated anthocyanins decreased postprandial glycaemia and insulinaemia. These effects have been detected in healthy study subjects after an acute intake of blueberries[196], lingonberries[197], black currants (glucose and insulin)[198,199], black currant juice fortified with crowberry (glucose and insulin)[200] and mixtures of berries (bilberries, black currants, cranberries, strawberries, lingonberries or cranberries) (only glycaemia for reference[201])[201–203].

There are several potential underlying mechanisms for the observed hypoglycaemic effect (Chapter 2.5). Anthocyanins may have affected the glycaemic and insulinaemic responses by inhibiting the carbohydrate digesting enzymes: salivary α-amylase[261], pancreatic α-amylase[264] and intestinal α-glucosidase[189,267]. Acylation significantly influences the inhibiting efficacy of anthocyanins on the maltase activity of intestinal α-glucosidase[267], and acylated anthocyanins are shown to be more effective than non-acylated anthocyanins in inhibiting pancreatic α-amylase[264]. Glucose absorption is inhibited as anthocyanins decrease the intestinal uptake of glucose[142,144,261,268,269]. Semi-purified purple potato extract[147] and phenolic extract of purple, red and white potatoes[215] may decrease the intestinal uptake. Anthocyanins increase the uptake of glucose to muscle and adipose tissues[266,272] possibly via increasing the expression and translocation of GLUT4[191,274,275], activating AMP kinases[278,279] and activating the insulin signalling pathway[274]. Furthermore, anthocyanins inhibit gluconeogenesis (hepatic glucose production)[195] via activating the hepatic AMPK[278,279,283] and increasing the secretion of insulin via enhancing the secretion of incretin hormones[198,271].

In addition to the mechanisms related to the parent anthocyanins, their degradation products and metabolites may contribute to the physiological effects as the bioavailability of anthocyanins as such is low.[26,31] The phenolic metabolites of anthocyanins may inhibit human salivary α-amylase and decrease the intestinal uptake of glucose.[261] Another mechanism may be related to the glycaemic index of the ingested potatoes in **Study I**, as potatoes with higher polyphenol content have a lower glycaemic index.[211] However, studies of these mechanisms with acylated anthocyanins are scarce. Therefore, more molecular-level studies investigating the underlying hypoglycaemic mechanisms of monoacylated methoxysubstituted anthocyanins should be conducted in the future to support the observations of the postprandial clinical trials reported here.

However, the observed hypoglycaemic effect may not have resulted only from the impact of the acylated anthocyanins of the purple potatoes. In **Study I**, the varietal differences *e.g.* in the polyphenol, (resistant) starch and vitamin C contents may have affected the glycaemic and insulinaemic responses. In **Study II**, the differences between the test meals in the chlorogenic acid and acetic acid concentrations may also have affected the results (see Chapter 5.6.1).

5.4 Postprandial inflammation markers

In **Study II**, the PPE affected some of the 92 investigated postprandial inflammation markers after one meal (**Table 12**). The levels of C-C motif chemokine 20 (CCL20, $p < 0.001$), fibroblast growth factor 19 (FGF-19, $p < 0.001$), eukaryotic translation initiation factor (4E-BP1, $p = 0.045$), C-C motif chemokine ligand 25 (CCL25, $p = 0.045$), interleukin-8 (IL-8, $p = 0.011$), oncostatin-M (OSM, $p = 0.005$) and transforming growth factor alpha (TGF-alpha, $p = 0.045$) were all increased by the study meal with a statistically significant difference between the meal types at 240 minutes. In addition, the levels of Fms-related tyrosine kinase (Fit3L, $p < 0.001$ and $p = 0.003$), monocyte chemotactic protein 1 (MCP-1, $p < 0.001$ and $p = 0.004$), matrix metalloproteinase 10 (MMP-10, $p < 0.001$ and $p = 0.031$), TNF receptor superfamily member 9 (TNFRSF9, $p < 0.001$ and $p = 0.013$) and TNF-related activation-induced cytokine (TRANCE, $p < 0.001$ and $p < 0.001$) were decreased 240 min after the control meal and study meal, respectively, compared with the fasting state and at 240 minutes.

The elevation of the pro-inflammatory cytokine interleukin-6 (IL-6) after a carbohydrate-rich meal in healthy volunteers, as reported in the literature[328], was confirmed in this study ($p < 0.001$). The study meal caused a smaller rise than the control meal without statistical significance. The insulin-like FGF-19 was elevated statistically significantly more after the study meal compared to the

control meal. FGF-19 has been reported to be potentially anti-diabetic as it decreases the glucose levels independently from insulin possibly by converting glucose to lactate in rodents.[329] It also increases the metabolic rate in high-fat fed mice[330], regulates the hepatic glucose homeostasis by suppressing gluconeogenesis[331] and induces the glycogen synthesis[332].

The effect of anthocyanin-rich foods on the postprandial inflammation markers of healthy volunteers (Chapter 2.4) have been detected *e.g.* with strawberries[236], cranberry juice[237] and red wine anthocyanins[238]. In the case of purple potatoes, the acute effect has not been studied earlier, but a six-week daily intake of 150 g of purple potatoes decreased the levels of IL-6 and CRP in comparison to white potatoes.[248] An *in vitro* study showed that purple potato extract decreased IL-8 in Caco-2 cells.[147] The molecular mechanisms behind these effects may involve the suppression of NF-κB.[147] As a high intake of glucose and fat is linked to postprandial inflammation[225], investigating the inflammation markers after one meal is essential for understanding the health effects of the food. However, the biological relevance of the observed changes in the inflammation markers nevertheless remains a study question for the future.

Table 12. The plasma inflammation markers at the fasting state and 240 min after the study and control meals analysed using the cDNA-based proximity extension multiplex immunoassay and qPCR. The values are means (n=17) using an arbitrary, semi-quantitative log2 scale. Variation is given as standard deviation (SD). Reprinted from the original publication **II**[326] with the permission of Elsevier.

	Control meal							Study meal							Control versus study meal					
	0 min		240 min					0 min		240 min					0 min			240 min		
Marker	Mean	SD	Mean	SD	*p*	*q*	ES	Mean	SD	Mean	SD	*p*	*q*	ES	*p*	*q*	ES	*p*	*q*	ES
Eukaryotic translation initiation factor 4E-binding protein 1	4.91	1.82	4.35	0.91				4.52	1.29	5.18	1.73							0.045	0.269	0.291
Adenosine deaminase	3.36	0.40	3.18	0.45	0.014	0.115	0.668	3.29	0.55	3.17	0.88									
Artemin	0.12	0.00	0.12	0.00				0.12	0.00	0.12	0.00									
Axin-1	1.58	0.74	1.52	0.65				1.72	0.64	1.64	0.81									
Beta-nerve growth factor	1.28	0.19	1.13	0.23	0.015	0.118	0.662	1.26	0.32	1.17	0.36									
Caspase-8	1.31	0.72	0.87	0.52				1.18	0.61	1.12	0.68									
C-C motif chemokine 3	3.77	0.39	3.56	0.40	0.004	0.055	0.813	3.83	0.54	3.68	0.46									
C-C motif chemokine 4	5.44	0.47	5.28	0.50	0.010	0.103	0.703	5.47	0.50	5.44	0.50									
C-C motif chemokine 11	8.24	0.72	8.13	0.68	0.013	0.113	0.382	8.17	0.65	8.16	0.55									
C-C motif chemokine 19	8.15	0.42	7.98	0.57				8.14	0.71	8.16	0.50									
C-C motif chemokine 20	4.48	0.69	4.06	0.59	0.002	0.041	0.875	4.52	0.89	4.48	0.80							<0.001	0.022	0.989
C-C motif chemokine 23	9.40	0.30	9.36	0.45				9.39	0.38	9.43	0.42									
C-C motif chemokine 25	5.48	0.80	5.21	0.90	<0.001	0.018	1.032	5.46	0.93	5.47	0.67							0.045	0.269	0.526
C-C motif chemokine 28	0.46	0.51	0.46	0.51				0.47	0.41	0.38	0.21									
Natural killer cell receptor 2B4	5.35	0.26	5.23	0.31	0.037	0.245	0.552	5.35	0.30	5.29	0.46									
CD40L receptor	8.87	0.34	8.79	0.25				8.91	0.41	8.99	0.38									
T cell surface glycoprotein CD5	4.42	0.27	4.20	0.41	0.001	0.027	0.935	4.39	0.42	4.30	0.40									
T cell surface glycoprotein CD6 isoform	3.72	0.40	3.47	0.40	0.001	0.023	0.961	3.73	0.48	3.54	0.43									
CUB domain-containing protein 1	1.54	0.39	1.38	0.47	0.009	0.101	0.403	1.52	0.46	1.46	0.44									
Macrophage colony-stimulating factor 1	7.17	0.21	7.01	0.23	0.013	0.113	0.673	7.18	0.27	7.14	0.28									
Cystatin D	5.31	0.52	5.17	0.57	0.023	0.177	0.608	5.22	0.54	5.15	0.51									

Marker	Control meal							Study meal							Control versus study meal					
	0 min		240 min					0 min		240 min					0 min			240 min		
	Mean	SD	Mean	SD	*p*	*q*	ES	Mean	SD	Mean	SD	*p*	*q*	ES	*p*	*q*	ES	*p*	*q*	ES
Fractalkine	5.07	0.55	4.82	0.41	0.030	0.216	0.578	5.09	0.40	4.96	0.59									
C-X-C motif chemokine 1	7.59	0.44	7.48	0.43				7.52	0.49	7.72	0.41									
C-X-C motif chemokine 5	9.73	0.84	9.62	0.71				9.74	0.88	9.83	0.73									
C-X-C motif chemokine 6	8.41	0.48	8.20	0.60	0.040	0.248	0.543	8.31	0.46	8.41	0.51									
C-X-C motif chemokine 9	6.95	0.93	6.66	0.96	0.002	0.030	0.503	6.85	1.04	6.70	0.81									
C-X-C motif chemokine 10	7.76	0.49	7.34	0.60	<0.001	0.016	1.053	7.72	0.69	7.45	0.65									
C-X-C motif chemokine 11	10.61	1.17	10.48	1.12				10.54	1.26	10.57	1.21									
Delta and Notch-like epidermal growth factor-related receptor	7.70	0.21	7.62	0.20				7.72	0.22	7.68	0.30									
Protein S100-A12	2.54	0.88	2.19	0.82				2.38	0.78	2.35	0.88									
Fibroblast growth factor 5	0.29	0.00	0.29	0.00				0.29	0.00	0.29	0.03									
Fibroblast growth factor 19	7.37	0.90	6.90	0.87	0.033	0.230	0.566	7.65	0.77	7.74	1.10							0.001	0.023	0.969
Fibroblast growth factor 21	2.93	1.53	2.61	1.38				2.99	1.48	2.71	1.29									
Fibroblast growth factor 23	1.78	0.77	1.43	0.73	0.003	0.042	0.480	1.81	0.93	1.59	0.54									
Fms-related tyrosine kinase 3 ligand	7.87	0.41	7.26	0.39	<0.001	<0.001	2.235	8.00	0.57	7.48	0.49	0.003	0.050	0.839						
Glial cell line-derived neutrotrophic factor	0.63	0.14	0.59	0.08				0.57	0.03	0.59	0.07									
Hepatocyte growth factor	6.65	0.17	6.48	0.25	0.011	0.103	0.697	6.65	0.32	6.62	0.29									
Interferon gamma	0.45	0.03	0.45	0.00				0.45	0.00	0.45	0.00									
Interleukin-2	0.49	0.00	0.49	0.00				0.49	0.00	0.49	0.00									
Interleukin-2 receptor subunit beta	0.79	0.00	0.79	0.00				0.79	0.00	0.79	0.00									
Interleukin-4	0.65	0.61	0.65	0.57				0.64	0.56	0.64	0.62									
Interleukin-5	1.04	0.79	1.03	0.79				1.00	0.67	0.97	0.65									
Interleukin-6	2.20	0.89	4.13	1.16	<0.001	0.001	0.715	2.13	0.85	3.74	1.18	<0.001	0.006	1.207						
Interleukin-7	1.82	0.38	1.71	0.31	0.004	0.056	0.807	1.79	0.37	1.84	0.29									
Interleukin-8	5.01	0.41	4.84	0.36	0.039	0.247	0.546	5.05	0.50	5.22	0.55							0.011	0.103	0.699
Interleukin-10	1.71	0.38	1.48	0.44	0.010	0.101	0.713	1.94	0.63	1.64	0.53									
Interleukin-10 receptor subunit alpha	0.45	0.33	0.40	0.33				0.46	0.37	0.55	0.57									
Interleukin-10 receptor subunit beta	6.40	0.27	6.19	0.29	0.003	0.050	0.833	6.34	0.23	6.22	0.43									
Interleukin-12 subunit beta	2.98	0.54	2.93	0.57				3.06	0.70	3.09	0.46									

Marker	Control meal							Study meal							Control versus study meal					
	0 min		240 min					0 min		240 min					0 min			240 min		
	Mean	SD	Mean	SD	*p*	*q*	ES	Mean	SD	Mean	SD	*p*	*q*	ES	*p*	*q*	ES	*p*	*q*	ES
Interleukin-13	0.46	0.00	0.46	0.00				0.46	0.00	0.46	0.00									
Interleukin-15 receptor subunit alpha	0.22	0.00	0.22	0.00				0.22	0.00	0.22	0.00									
Interleukin-17A	0.44	0.44	0.40	0.41				0.44	0.38	0.40	0.41									
Interleukin-17C	0.77	0.23	0.65	0.12				0.80	0.35	0.62	0.07	0.036	0.245	0.586						
Interleukin-18	6.79	0.55	6.68	0.62				6.83	0.73	6.88	0.49									
Interleukin-18 receptor 1	5.94	0.38	5.85	0.46				6.02	0.51	5.97	0.44									
Interleukin-20	0.58	0.51	0.57	0.48				0.51	0.20	0.46	0.00									
Interleukin-20 receptor subunit alpha	0.37	0.31	0.37	0.34				0.38	0.38	0.38	0.37									
Interleukin-22 receptor subunit alpha 1	1.13	0.00	1.13	0.00				1.13	0.00	1.13	0.00									
Interleukin-24	1.08	0.00	1.08	0.00				1.08	0.00	1.08	0.00									
Interleukin-33	0.81	0.00	0.81	0.00				0.81	0.00	0.87	0.25									
Latency-associated peptide transforming growth factor beta 1	6.38	0.28	6.26	0.25				6.38	0.35	6.53	0.72									
Leukemia inhibitory factor	0.69	0.51	0.68	0.54				0.68	0.49	0.63	0.52									
Leukemia inhibitory factor receptor	2.20	0.23	2.08	0.26				2.24	0.27	2.18	0.42									
Monocyte chemotactic protein 1	9.94	0.24	9.44	0.33	<0.001	0.006	1.205	10.01	0.39	9.58	0.42	0.004	0.055	0.818						
Monocyte chemotactic protein 2	7.41	0.72	7.28	0.67				7.38	0.49	7.43	0.64									
Monocyte chemotactic protein 3	1.89	0.44	1.81	0.44				1.96	0.39	1.97	0.50									
Monocyte chemotactic protein 4	5.28	0.85	5.06	0.65	0.023	0.177	0.611	5.20	0.72	5.21	0.69									
Matrix metalloproteinase 1	10.23	0.97	10.01	0.91	0.006	0.064	0.777	10.09	0.98	10.38	0.99									
Matrix metalloproteinase 10	5.35	0.56	4.99	0.57	<0.001	<0.001	1.739	5.25	0.68	4.74	0.90	0.031	0.216	0.321						
Neurturin	0.40	0.30	0.38	0.31				0.34	0.13	0.36	0.15									
Neurotrophin-3	0.95	0.42	0.91	0.33				0.94	0.41	0.98	0.45									
Osteoprotegerin	9.61	0.31	9.49	0.30	0.012	0.110	0.685	9.59	0.35	9.53	0.27									
Oncostatin-M	1.16	1.02	0.78	0.90	0.008	0.087	0.415	1.02	1.02	1.46	1.08							0.005	0.056	0.446
Programmed cell death 1 ligand 1	3.43	0.29	3.17	0.38	<0.001	0.022	0.539	3.37	0.33	3.28	0.55									
Stem cell factor	9.51	0.22	9.47	0.22				9.46	0.22	9.43	0.41									
SIR2-like protein 2	1.69	0.92	1.43	0.54				1.57	0.66	1.82	1.04									
Signalling lymphocytic activation molecule	0.92	0.64	0.83	0.59				0.97	0.64	0.90	0.74									

Marker	Control meal							Study meal							Control versus study meal					
	0 min		240 min					0 min		240 min					0 min			240 min		
	Mean	SD	Mean	SD	*p*	*q*	ES	Mean	SD	Mean	SD	*p*	*q*	ES	*p*	*q*	ES	*p*	*q*	ES
Sulfotransferase 1A1	3.02	1.20	3.01	1.26				3.06	1.06	3.10	1.22									
STAM-binding protein	3.63	0.95	3.23	0.73				3.46	0.76	3.47	1.06									
Transforming growth factor alpha	1.90	0.33	1.80	0.28				1.94	0.35	2.04	0.39							0.045	0.269	0.527
Tumor necrosis factor	0.43	0.00	0.43	0.00				0.43	0.00	0.43	0.00									
TNF-beta	3.45	0.34	3.33	0.24	0.030	0.216	0.577	3.43	0.51	3.32	0.51									
TNF receptor superfamily member 9	5.64	0.34	5.30	0.37	<0.001	0.001	1.463	5.65	0.48	5.34	0.42	0.013	0.113	0.674						
TNF ligand superfamily member 14	3.44	0.36	3.13	0.43	0.005	0.056	0.798	3.37	0.51	3.27	0.75									
TNF-related apoptosis-inducing ligand	7.75	0.25	7.56	0.26	0.011	0.103	0.701	7.74	0.37	7.51	0.52									
TNF-related activation-induced cytokine	4.25	0.56	3.75	0.64	<0.001	0.006	1.190	4.27	0.76	3.77	0.73	<0.001	0.006	1.218						
Thymic stromal lymphopoietin	0.67	0.00	0.67	0.01				0.70	0.10	0.67	0.00									
TNF (Ligand) superfamily, member 12	8.66	0.24	8.53	0.28	0.039	0.247	0.546	8.66	0.30	8.60	0.39									
Urokinase-type plasminogen activator	9.90	0.25	9.72	0.25	0.001	0.023	0.960	9.93	0.36	9.76	0.37									
Vascular endothelial growth factor A	8.07	0.25	7.84	0.29	<0.001	0.010	1.126	8.06	0.34	8.01	0.28									

q-values, the Benjamini–Hochberg corrected *p*-values

ES, the effect size (Cohen's *d* or *r*, depending on normality of the data)

5.5 Phenolic metabolites

5.5.1 Anthocyanins

The ingested acylated anthocyanins from the purple potato extract did not survive as such into the plasma or urine (**III**). Six anthocyanin degradants were detected in the urine samples (**Figure 11**) of which cyanidin-3-*O*-glucoside was identified with a standard compound, and cyanidin-feruloyl-rutinoside, malvidin-rutinoside, petunidin-glucoside, peonidin-glucoside and peonidin-glucuronide are tentatively identified. This data shows that the acyl group and the rutinose are hydrolysed from the acylated anthocyanins, but in contrast to the non-acylated anthocyanins, the glucose moieties of acylated anthocyanins may remain attached. The detected anthocyanin degradants were persistent in urine as reported earlier with non-acylated anthocyanins, suggesting enterohepatic circulation of the degradants.[156] As cyanidin derivatives were detected, it is proposed that the methoxysubstituted anthocyanidins may be interconverted to cyanidin, or the minor cyanidin-based anthocyanins in the purple potatoes are more bioavailable than the methoxysubstituted ones.

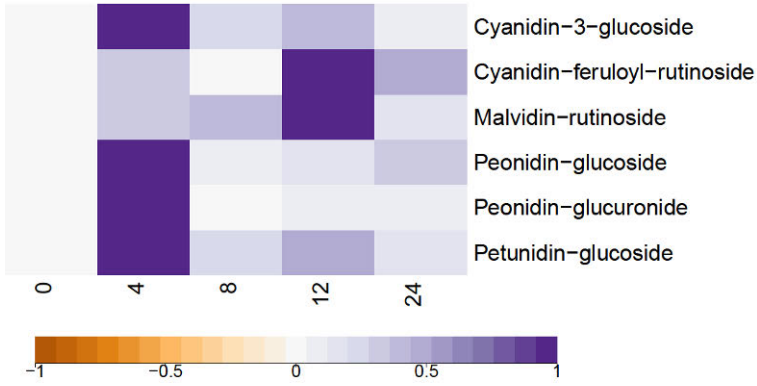


Figure 11. Tentatively identified anthocyanins from urine samples after a potato meal rich in acylated anthocyanins (**III**). The columns represent time in hours; fasting state (0), 0–4, 4–8, 8–12 and 12–24 h postprandially. The response is a *delta* variable presenting change between the control meal (orange colour) and the study meal (purple colour), standardised to the range of [-1, 1].

The previous studies show that the bioavailability of anthocyanins is low. The majority of studies report urinary recovery less than 1%[34,178–180] or 2%[158] (see also **Table 2**, Chapter 2.2.2). Acylation decreases the bioavailability of anthocyanins even further; for example, the mainly diacylated cyanidin diglucosides of red cabbage are recovered four times less whereas the monoacylated cyanidin xylosides of purple carrots are recovered 14 times less than the non-acylated anthocyanins of these foods.[28,30] In contrast to the results presented here, some previous studies have detected structurally different mono- and diacylated anthocyanins from the biological samples of healthy study volunteers[28,30,140,160,176] but not after a meal of purple potatoes[175] (Chapter 2.2.2).

5.5.2 Phenolic metabolites and conjugates

Altogether 28 and 14 phenolic metabolites identified with a corresponding standard compound were found in the urine and plasma, respectively (**Figures 12–13**). Of the detected metabolites in urine, 19 were elevated statistically differently after the study meal, and x in plasma. Urinary hippuric acid was the most abundant; it reached an increase of 139 μM/mM creatinine from the control levels after the study meal. In urine, monomethoxysubstituted vanillic acid, corresponding to the B-ring of petunidin, and dimethoxysubstituted sinapic acid, corresponding to the B-ring of peonidin, were detected. *O*-Demethylation of the B-ring may occur, as the potato anthocyanins are mainly methoxysubstituted, but catechol and protocatechuic acid and its derivatives, which contain a dihydroxylated benzene ring, were detected.

As literature is limited about the phenolic metabolites of acylated anthocyanins, these results may be compared to the *in vivo* studies conducted with foods rich in non-acylated anthocyanins such as aronia[33], bilberries[34], bilberry-lingonberry puree[32], blueberries[36,156], cranberries[35,38], elderberries[40] and raspberries[37,184] and grapes containing a mix of non-acylated and acylated anthocyanins[90,155]. One study tentatively identified some metabolites after an acute meal of purple potatoes, but did not include a control arm.[175] These results show that like non-acylated anthocyanins, also acylated anthocyanins are degraded into phenolic metabolites *in vivo* even though they are chemically more stable. The extent of the degradation of acylated anthocyanins into phenolic metabolites was large. Most of the detected metabolites were identified here for the first time after a meal rich in acylated anthocyanins except for (iso)ferulic acid, dihydroferulic acid, dihydrocaffeic acid sulfate, homovanillic acid/dihydrocaffeic acid and hippuric acid which were detected earlier by Tsang et al. after a meal of purple potatoes.[175] As with non-acylated anthocyanins, also phenolic metabolites of acylated anthocyanins may be further conjugated in phase II metabolism.

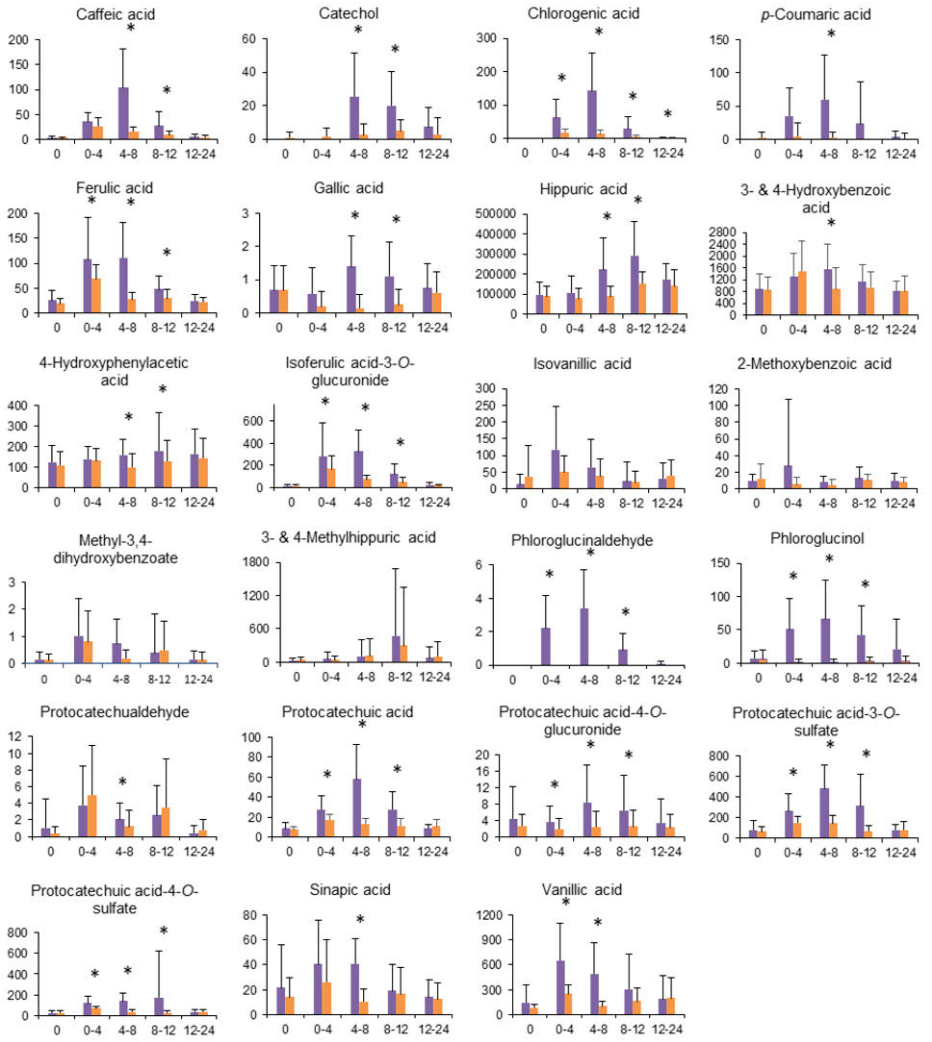


Figure 12. The phenolic metabolites (**III**) identified and quantified in the urine samples of 17 healthy men. The values are given as concentrations (nM/mM creatinine) with standard deviations in the fasting state (0-point) and at different time periods after the meal in hours. Asterisks show the statistical differences ($p < 0.05$) between the study (purple bars) and control (orange bars) meal samples at the corresponding time period.

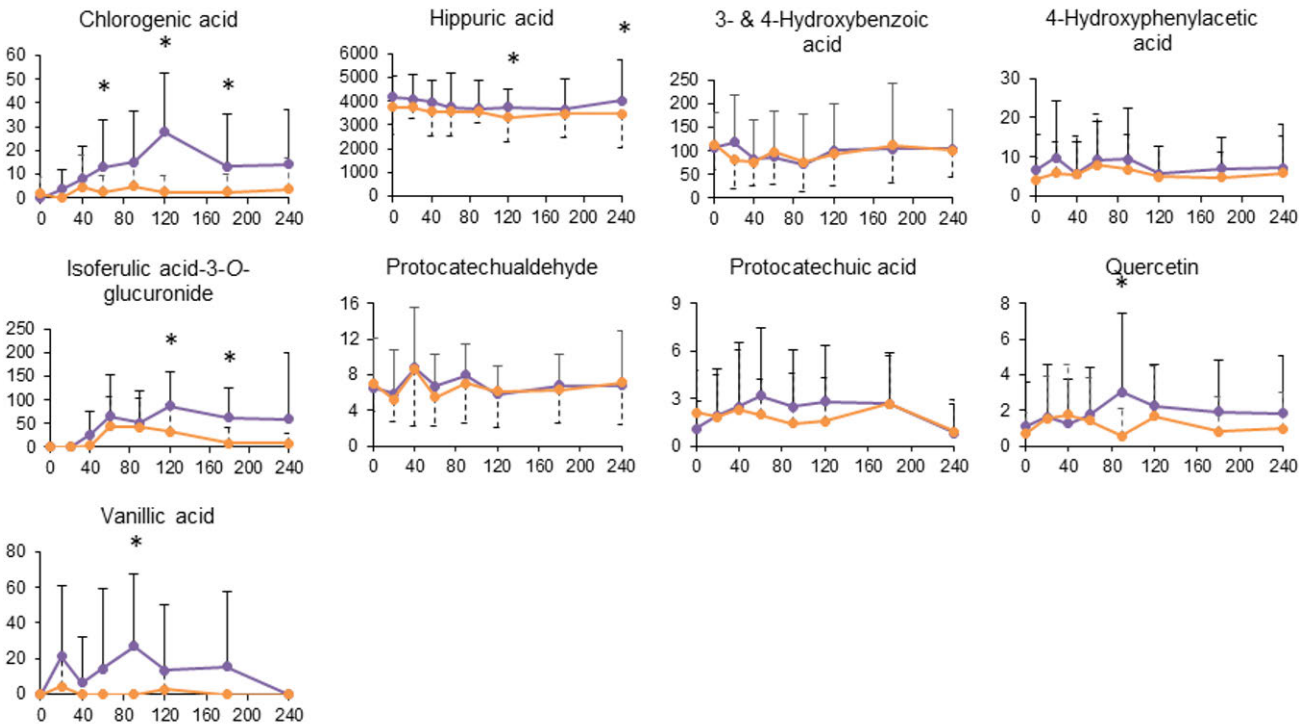


Figure 13. The phenolic metabolites (**III**) identified and quantified in the plasma samples of 17 healthy men. The values are given as concentrations (nM) with standard deviations in the fasting state (0-point) and at different time points after the meal in minutes. Asterisks show statistical difference ($p < 0.05$) between the study (purple lines) and control (orange lines) meal samples at the corresponding time point.

Speculating the possible *in vivo* degradation mechanism of acylated anthocyanins requires mirroring these results to the chemistry and metabolism of non-acylated anthocyanins and to the available *in vitro* studies regarding acylated anthocyanins. As described in detail in Chapter 2.2.1 and 2.2.2, the rapid hydrolysis of non-acylated anthocyanins under physiological environment is initiated already in the mouth.[118,120,120] The formed unstable aglycones undergo ring C fission via α-diketone leading to the formation of a phenolic acid (expected B-ring degradant) and an aldehyde (expected A-ring degradant)[109] which are further subjected to phase I and phase II reactions[158,159]. Acylation, however, protects the anthocyanins from the digestive processes of gastrointestinal tract.[121] A study conducted with an *in vitro* gastrointestinal model showed that a major part of the anthocyanins of purple potatoes (72%) may reach the colon which indicates enhanced stability in comparison to the acylated, dihydroxysubstituted anthocyanins of carrots (45%).[147] Another *in vitro* study showed that the gut microbiota may break down the mono- and diacylated pelargonidin sophoroside glucosides to phenolic metabolites.[109] The active role of gut microbiota is also substantiated by the detected late peak time points of the phenolic metabolites in this study. Therefore, the proposed degradation mechanism of acylated anthocyanins of purple potatoes in healthy human volunteers occurs first by enzymatic hydrolysis of the acyl and glycosyl groups followed by enzymatic and/or spontaneous ring-C fission of the unstable anthocyanidins leading to phenolic metabolites which may then be further subjected to phase I and II reactions (**Figure 14**).

Some of the observed phenolic metabolites may have originated from the hydroxycinnamic acids present in the PPE in addition to the acylated anthocyanins. For example, caffeic acid was present in minor amounts in the ingested PPE. Chlorogenic acid, abundant in the PPE, was detected also in the plasma and urine samples, and it may partially break down to caffeic acid.

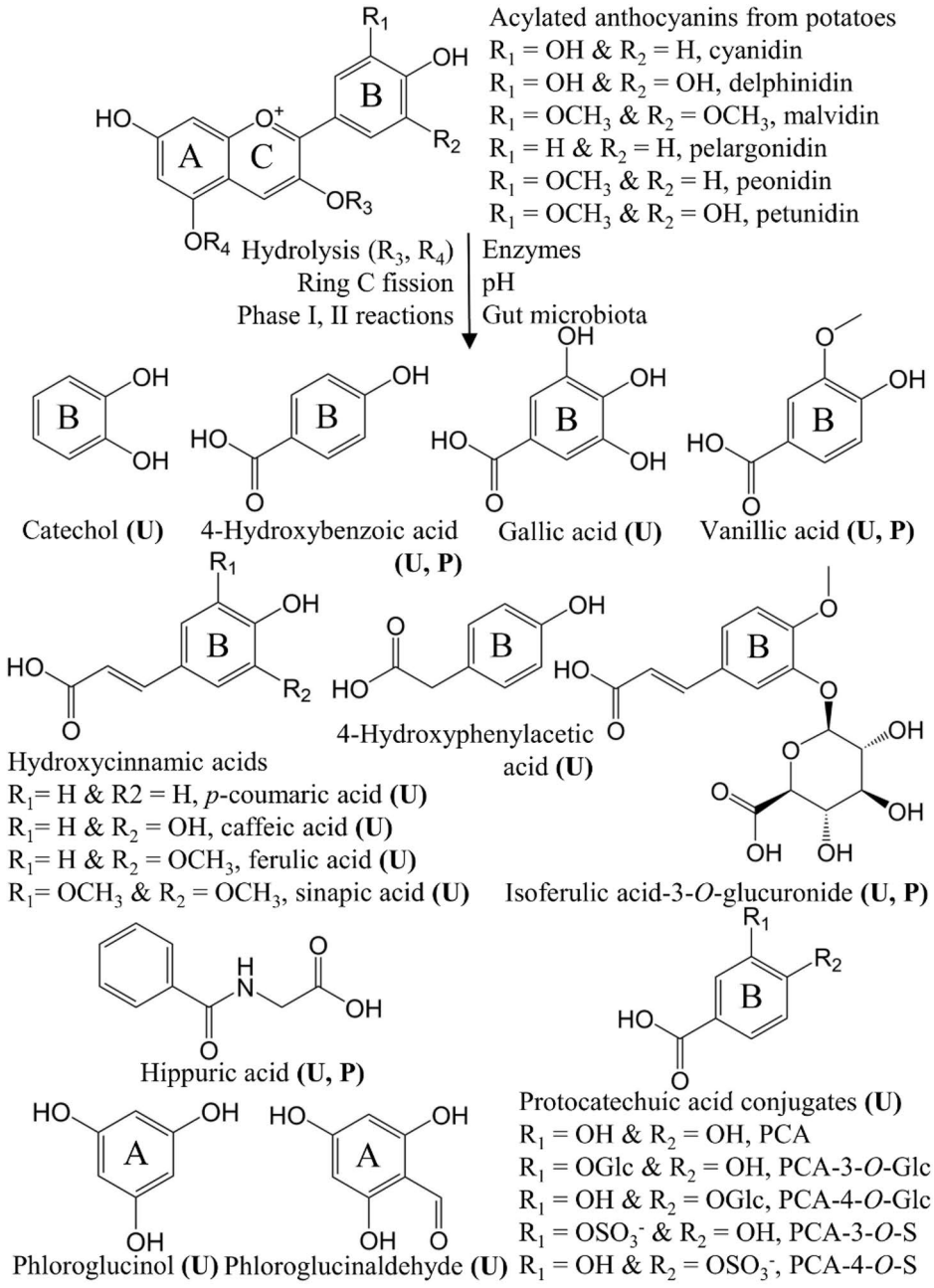


Figure 14. Phenolic metabolites found in the urine **(U)** and plasma **(P)** of healthy men after a meal of yellow potatoes supplemented with anthocyanin-rich purple potato extract. PCA, protocatechuic acid; glc, glucuronide; s, sulfate. R_3 and R_4 may carry a glycoside group of which the one in R_3 may be acylated.

5.6 General discussion

5.6.1 Limitations and strengths of the studies

Clinical study designs. Designing a clinical trial requires balancing the available resources and the end-points of the study. The trials conducted here were small-scale studies, which may be perceived as a limitation. However, these trials were the first steps in investigating the postprandial hypoglycaemic effect of purple potatoes and their anthocyanins in healthy men, and yet the number of participants exceeded the requirement of the power calculations. As these trials gave evidence of the hypoglycaemic effects of purple potatoes and their acylated anthocyanins, the next step is to organise large-scale studies to validate these results. A larger number of volunteers would represent the population better, leading to a reduced effect of inter-individual variation and chance. As a strength, the trials organised here were single-blinded, randomised repeated-measures cross-over studies, *i.e.* each volunteer acted as their own control. Comparing to a parallel design, cross-over design decreases the effect of inter-individual variation and thus does not require a similarly large sample size.

The baseline characteristics of the volunteers were carefully controlled with inclusion and exclusion criteria, and by asking the volunteers to follow the strict instructions for diet and exercise. The study participants were asked to refrain from exercise as it decreases postprandial glycaemia[333] by enhancing the translocation of GLUT4 in the skeletal muscle cell membranes[334]. The study diet was low in flavonoids and fibre to minimise their effect on the postprandial responses and metabolites. The anthocyanin metabolites are persistent in the human biofluids even after five days of restricted diet[32,156] and thus this was controlled for by also collecting fasting state samples. As a limitation, diaries of diet and exercise were not collected which would have given information about the compliance of the volunteers with the restrictions of the study. Instead, the volunteers were simply asked to confirm their compliance. The study design, sampling and study diet were already challenging for the volunteers and the diaries would have burdened them even more. As another limitation, the volunteers were all men as the phase of the menstruation cycle of woman volunteers would have required a more careful control due to its effect on blood glucose homeostasis.[335]

Test meals. As strengths, the amount of anthocyanins given in both clinical trials may be reached in an everyday diet and the chemical compositions of the test meals were carefully characterised which is often a major limitation in postprandial trials. In **Study I**, the test meals were served as steam-cooked mashed potatoes, which is a typical way of consuming potatoes. However, two different potato cultivars (yellow and purple) were used and therefore

standardising the nutrient content was not possible. This leads to differences between the study and control meals *e.g.* in vitamin C, (resistant) starch and phenolic compounds. This limitation was overcome in **Study II**, in which both the control and the study meal contained the same steam-cooked yellow potato mash, and the study meals were supplemented with a purple potato extract rich in acylated anthocyanins. Considering the phenolic metabolite profiling, metabolite studies are often limited by the use of a whole food or a mixed-type meal, leading to uncertainty as to where a detected metabolite originated. Here, this was controlled for by using the PPE instead of purple potatoes, even though this leads to forgoing the effect of the potato matrix on absorption and metabolism.

As another limitation, acetic acid, which was used to stabilise the pH-dependent anthocyanins during the extraction process of purple potatoes, and chlorogenic acid, which is a common ester of caffeic acid and quinic acid in potatoes, were abundant in the extract and thus may have affected the postprandial responses. Vinegar may decrease postprandial glycaemia, possibly resulting from the effect of low pH on enzyme activities.[336,337] To control for the effect of acetic acid, both the control and study meals were adjusted to the same pH (4). However, **Study I** gave evidence of the hypoglycaemic effect of purple potatoes compared to the yellow ones without the acetic acid. Chlorogenic acid, present in the PPE, may also have hypoglycaemic effects[338] and may be degraded after ingestion to caffeic acid, which is one of the investigated phenolic metabolites of acylated anthocyanins in **Study III**. Furthermore, the bilberry meal set as a positive control meal in **Study I**, was prepared using processed potato starch containing possibly more readily available carbohydrates, whereas the potato meals may have contained also resistant starch. Therefore, a direct comparison between the postprandial effects of non-acylated and acylated anthocyanins cannot be made on the basis of **Study I**.

Analytical methods. The chromatographic separation of acylated potato anthocyanins was improved in **Study II** from that of **Study I**, enabling better identification. However, the identification was based on high-resolution UHPLC-MS/MS analyses and literature instead of nuclear magnetic resonance spectroscopy (NMR) which is able to reveal the exact structure of a purified compound. The available NMR studies were, however, used in the identification.

Metabolism of reactive anthocyanins is an extensive multiorgan chain of events leading to relatively low concentrations of a number of metabolites and their conjugates for different time periods in complex biological samples. This creates a need for high-quality sample purification and concentration techniques, well-established analytical methods, modern sensitive analytical instrumentation and advanced data processing skills to handle a large amount of data. In **Study III**, a modern state-of-art mass spectrometer was used with the capacity to screen

a wide array of compounds without compromising the data points per peak. The SPE method and the analytical method were not optimised for acylated anthocyanins as the reference compounds were not available and the bioavailability of acylated anthocyanins has been reported to be extremely low[28,30,140]. The methods were, however, great for the non-acylated anthocyanins and phenolic metabolites. Additionally, using a targeted approach instead of an untargeted one restricts the detected metabolites to those that are explicitly searched for but yields a sufficient level of sensitivity required to accomplish this type of analysis.

The number of standard compounds needed for metabolite identification is high, yet they are expensive and, especially in the case of conjugates, often not available commercially. Here, many of the interesting metabolites were synthesised but still a significant number of the interesting ones were not available, leading to tentative identifications. Lastly, large inter-individual variations in phenolic metabolites was detected similar to the results in the literature[33,35,36,40]. This may be partly due to individual gut microbiota compositions which were not further investigated in this thesis.

5.6.2 Relevance and significance of the research

Before the experimental part of this thesis, clinical trials investigating the postprandial effects of anthocyanins have mainly been conducted with foods and extracts rich in non-acylated anthocyanins. Therefore, these studies provide novel information about the postprandial effects and metabolism of purple potatoes and their monoacylated and mainly methoxysubstituted anthocyanins. Acylated anthocyanins provide new food development opportunities for the food industry as they are more stable than the non-acylated ones.[21] The clinical trials in this thesis gave evidence that consuming purple potatoes and their anthocyanin-rich extract added to yellow potatoes decrease the postprandial blood glucose and insulinaemia in comparison to yellow potatoes; this promotes their use as a part of a versatile, healthy diet and in different applications of food industry. The consumption of purple potatoes is still limited in many countries outside South America, such as Finland, but nevertheless, potatoes in general are sustainable, ecological and a local food crop[50] widely consumed and cultivated all over the world. Consuming purple potatoes would increase the daily intake of anthocyanins which is, in Europe and Australia, acquired mainly from the consumption of berries, fruits and red wine[14–16] with limited availability and use. Investigating purple potatoes in Finland is reasonable, as the amount of anthocyanins in potato tubers increases in the northern locations due to the cold temperature and the long light period during the growing season.[59,62]

5.6.3 Future prospects

This PhD thesis supports the continuation of investigations into acylated anthocyanins in the future despite their observed low bioavailability in their original form, and more research into the study questions raised on the basis of these results. In the future, the optimal bioactive dose and the structure-activity relationships should be assessed, and the effect should be studied of gut microbiota, food matrix, cooking methods, potato variety and other dietary compounds on bioactivity and bioavailability. Using labelled acylated anthocyanins would benefit the future clinical trials to link the metabolites with the parent anthocyanins. The molecular mechanisms behind the bioactivity of pigmented potatoes and acylated anthocyanins should be further investigated; especially the physiological effects of the phenolic metabolites. Long-term intervention studies also involving women should be conducted to assess the effect of pigmented potatoes and acylated anthocyanins as part of an everyday diet, and the clinical endpoints broadened to include lipid metabolism and long-term inflammation. Furthermore, more studies are needed to harness the potential of acylated anthocyanins in food development applications for the food industry.

6 SUMMARY AND CONCLUSION

The experimental part of this thesis studied the effect of acylated anthocyanins on human postprandial carbohydrate metabolism and inflammation. The model food rich in acylated anthocyanins used for these studies was a purple potato variety, *Solanum tuberosum* L. 'Synkeä Sakari', as potatoes have a high glycaemic index and are consumed all over the world. Synkeä Sakari potatoes provided a rich source of anthocyanins of which 99 % were acylated. The major anthocyanins were methoxysubstituted petunidin and peonidin derivatives.

These results give clinical evidence that the consumption of purple potatoes rich in acylated anthocyanins decrease the highest peak of postprandial glucose. Frequent high blood glucose peaks are known to be detrimental and over years, they contribute to the development of metabolic disorders. When the two different potato varieties, a purple and a yellow one, were studied, the hypoglycaemic effect may have been affected by the varietal differences in *e.g.* vitamin C content and starch composition in addition to the differences in the polyphenol content. Thus, to investigate the effect of the acylated anthocyanins on glycaemia, the anthocyanins of the purple potatoes were extracted, and a follow-up clinical trial was organised. The anthocyanin-rich purple potato extract decreased the highest postprandial blood glucose and insulin peaks and prevented the concentrations from decreasing below the fasting state levels which occurred after the control meal. The extract also modified the postprandial inflammation markers; for example, the insulin-like hormone FGF-19, was elevated in comparison to the control meal.

As the bioavailability of acylated anthocyanins has been reported to be extremely low and the metabolism of acylated anthocyanins has not previously been investigated thoroughly in healthy human volunteers, in this study the metabolites of acylated anthocyanins were screened from the plasma and urine samples collected in the second clinical intervention. The results showed that the acylated anthocyanins may not be absorbed as such into the systemic circulation nor excreted via urine but instead a large number of phenolic metabolites was detected. It may be speculated that the acute physiological effects of acylated anthocyanins may be affected by the intact, non-absorbed acylated anthocyanins in the gastrointestinal tract and/or the phenolic metabolites forming shortly after the meal. However, majority of the detected phenolic metabolites peaked later than the observed physiological effects, possibly due to colonic degradation, and therefore may not have affected the observed postprandial health outcomes. The effect of the metabolites on postprandial and long-term glycaemia and inflammation remains, however, as a follow-up research question for the future.

Overall, these results provide information on the postprandial health effects and metabolism of anthocyanins monoacylated with hydroxycinnamic acids in healthy human volunteers. The results support the usage of acylated anthocyanins in different food development applications from the perspective of the health effects. For food industrial purposes, acylated anthocyanins may be extracted from the easily-cultivated and ecological purple potatoes and their peels from food industrial waste streams using food-grade processes. These results promote the use of purple potatoes as a dietary source of anthocyanins in addition to berries and fruits as a part of a versatile, everyday diet, and suggest complementing the more commonly used yellow potatoes with purple ones to obtain additional nutritional value.

ACKNOWLEDGEMENTS

The work for this thesis was mainly conducted at the Food Chemistry and Food Development unit of the Department of Biochemistry, University of Turku, Finland. The bioanalytical UHPLC–MS/MS metabolite study (III) was conducted at the Quadram Institute Biosciences, Norwich, United Kingdom.

I am very thankful to the Doctoral Programme in Molecular Food Sciences (DPMLS) of the University of Turku Graduate School (UTUGS) for my four years of full-time employment. I wish to warmly thank the Raisio Research Foundation, the Niemi Foundation, the Magnus Ehrnrooth Foundation, the Orion Research Foundation, DPMLS/UTUGS and the Finnish Society for Nutrition Research for their generous grants enabling the experiments and opportunity to travel.

I wish to express my sincere gratitude to my supervisors, Professor Baoru Yang and Associate Professor Kaisa Linderborg, for this great opportunity, support and guidance! Kaisa, my special thanks for encouraging me to conduct a PhD from the very early stages of my undergraduate studies and for inducting me into the organisation for clinical trials. Professor Emeritus Heikki Kallio, thank you for introducing me to anthocyanins and for setting an inspiring example. Assistant Professor Maaria Kortesniemi, thank you for your support, great discussions and for your friendship! Thank you, Professor Matti Viitanen and PhD Reetta Satokari, for participating in my advisory committee and giving me wise advice. Matti, thank you also for your excellent collaboration in the clinical interventions. Professor Emerita Kaisa Poutanen and Dr Ana Rodriguez-Mateos, thank you for conducting the official review and expressing your constructive criticism of this PhD thesis.

PhD Mikko Griinari, thank you for providing the purple potatoes, and Professor Tuomo Sainio and PhD Jari Heinonen, thank you for the purple potato extract. My warm thanks to Dr Paul Kroon, Mark Philo, Jasmine Percival and all the lab members of the Kroon group from the Quadram Institute Biosciences! Dr Kroon, I am the most grateful for inviting me to visit your research group for six months, for your support and inspiration. Mark, thank you for all the good laughs, and teaching me bioanalytics from the sample preprocessing to the UHPLC–MS/MS analyses and data processing. Jasmine, thank you for sharing the experience and for your friendship! Sanna Himanen, warm thanks for your excellent assistance in both clinical trials and for your friendship. Marika Kalpio and PhD Anssi Vuorinen, thank you for your guidance in Study I!

Before my PhD journey, I was working in the Instrument Centre of the University of Turku and Åbo Akademi, in which I was able to create the foundation for my analytical skills in both NMR and LC-MS. Jari Sinkkonen,

I'm most grateful to you for your friendship, teaching me NMR and encouraging me to continue academic research. Maarit Karonen, thank you for giving me the sparkle for LC-MS and for your friendship. Thank you my dear friends Aino-Liisa Alanne and Tahereh Jafari (the Powerpuff girls!), Elina Puljula and Lassi Raivonen for all the peer support, discussions, laughs and learning experiences!

I wish to acknowledge all the previous and present colleagues in the Food Chemistry and Food Development unit – you have all participated in my journey towards my PhD as teachers, mentors and friends! Especially Mika Kaimainen, Jukka-Pekka Suomela and Marko Tarvainen are to be thanked for valuable advice with instrument problems, and Salla Hakkola, Niina Kelanne, Amruta Kulkarni, Lotta Nylund, Anu Nuora and Anna Puganen for fun breaks and peer support. My heartfelt thanks and warm hugs to Heikki Aisala, Gabriele Beltrame and Niko Markkinen for your friendship and support both at work and outside. You have inspired me, believed in me and brought me so much joy through these years. Long live the Kultapossukerho! Shuxun Liu, Alexis Marsol, Raghunath Pariyani and Ye Tian, thank you for sharing the office and for all discussions! The technical and administrative personnel of the Department of Biochemistry is warmly thanked: Anu Hirvensalo, Mirva Jalo, Heli Kuusela, Leena Neuvonen, Tapio Ronkainen and Jani Sointusalo. I wish to thank the Food Chemistry students Linda Intonen, Maisa Lintala and Aino Tarkkio for your help in the clinical trials and Laura Aaltonen for your help in the anthocyanin analyses.

I wish to express my deepest gratitude to my parents Ritva and Tarmo for your support and encouragement, and thank you, my sister Sarianna, for setting the bar high ever since we were little. Kiitän Sirpa-mummuani ja muistan edesmenneitä Martti-vaariani ja Taimi-mummuani lämpimästi siitä, että he esimerkillään ja yhdessä tekemällä opettivat minut pienestä pitäen arvostamaan ahkeraa työntekoa, maanviljelyä ja suomalaisia marjoja. My parents-in-law Ilkka and Pirjo, thank you for all the discussions and warming up the sauna by the lake whenever we visited you. I wish to thank all my friends, especially Jonna, Susanna and Tiina, the scouts of Kaarinan Ristiritarit and Naurulokit from the Value Based Leadership 5 Finland, for many great adventures and patience when I was busy in the lab. My warmest and sincerest thanks belong to my beloved husband Tuomas. Your love, endless support and great sense of humour mean the world to me.

Turku, November, 2020

REFERENCES

1. Meessen, E. C. E.; Warmbrunn, M. V.; Nieuwdorp, M.; Soeters, M. R. Human postprandial nutrient metabolism and low-grade inflammation: a narrative review. *Nutrients*, **2019**, *11* (12), 3000.

2. World Health Organization. Definition of Health. Preamble to the Constitution of WHO as adopted by the International Health Conference, New York, 19 June - 22 July 1946; signed on 22 July 1946 by the representatives of 61 States (Official Records of WHO, no. 2, p. 100) and entered into force on 7 April 1948.

3. Ruiz-Núñez, B.; Pruimboom, L.; Dijck-Brouwer, D. A. J.; Muskiet, F. A. J. Lifestyle and nutritional imbalances associated with Western diseases: causes and consequences of chronic systemic low-grade inflammation in an evolutionary context. *The Journal of Nutritional Biochemistry*, **2013**, *24* (7), 1183–1201.

4. Ceriello, A.; Esposito, K.; Piconi, L.; Ihnat, M. A.; Thorpe, J. E.; Testa, R.; Boemi, M.; Giugliano, D. Oscillating glucose is more deleterious to endothelial function and oxidative stress than mean glucose in normal and type 2 diabetic patients. *Diabetes*, **2008**, *57* (5), 1349–1354.

5. Monnier, L. Is postprandial glucose a neglected cardiovascular risk factor in type 2 diabetes? *European Journal of Clinical Investigation*, **2000**, *30* (s2), 3–11.

6. Hu, F. B.; Manson, J. E.; Stampfer, M. J.; Colditz, G.; Liu, S.; Solomon, C. G.; Willett, W. C. Diet, lifestyle, and the risk of type 2 diabetes mellitus in women. *New England Journal of Medicine*, **2001**, *345* (11), 790–797.

7. Tuomilehto, J.; Lindström, J.; Eriksson, J. G.; Valle, T. T.; Hämäläinen, H.; Ilanne-Parikka, P.; Keinänen-Kiukaanniemi, S.; Laakso, M.; Louheranta, A.; Rastas, M.; Salminen, V.; Aunola, S.; Cepaitis, Z.; Moltchanov, V.; Hakumäki, M.; Mannelin, M.; Martikkala, V.; Sundvall, J.; Uusitupa, M. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *New England Journal of Medicine*, **2001**, *344* (18), 1343–1350.

8. Ford, E. S.; Mokdad, A. H. Fruit and vegetable consumption and diabetes mellitus incidence among U.S. adults. *Preventive Medicine*, **2001**, *32* (1), 33–39.

9. Guo, X.; Yang, B.; Tan, J.; Jiang, J.; Li, D. Associations of dietary intakes of anthocyanins and berry fruits with risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective cohort studies. *European Journal of Clinical Nutrition*, **2016**, *70* (12), 1360–1367.

10. Guo, H.; Ling, W. The update of anthocyanins on obesity and type 2 diabetes: experimental evidence and clinical perspectives. *Reviews in Endocrine & Metabolic Disorders*, **2015**, *16* (1), 1–13.

11. Hanhineva, K.; Törrönen, R.; Bondia-Pons, I.; Pekkinen, J.; Kolehmainen, M.; Mykkänen, H.; Poutanen, K. Impact of dietary polyphenols on carbohydrate metabolism. *International Journal of Molecular Sciences*, **2010**, *11* (4), 1365–1402.

12. Wedick, N. M.; Pan, A.; Cassidy, A.; Rimm, E. B.; Sampson, L.; Rosner, B.; Willett, W.; Hu, F. B.; Sun, Q.; van Dam, R. M. Dietary flavonoid intakes and risk of type 2 diabetes in US men and women. *The American Journal of Clinical Nutrition*, **2012**, *95* (4), 925–933.

13. Williamson, G. The role of polyphenols in modern nutrition. *Nutrition Bulletin*, **2017**, *42* (3), 226–235.

14. Zamora-Ros, R.; Knaze, V.; Luján-Barroso, L.; Slimani, N.; Romieu, I.; Touillaud, M.; Kaaks, R.; Teucher, B.; Mattiello, A.; Grioni, S.; Crowe, F.; Boeing, H.; Förster, J.; Quirós, J. R.; Molina, E.; Huerta, J. M.; Engeset, D.; Skeie, G.; Trichopoulou, A.; Dilis, V.; Tsiotas, K.; Peeters, P. H. M.; Khaw, K.-T.; Wareham, N.; Bueno-de-Mesquita, B.; Ocké, M. C.; Olsen, A.; Tjønneland, A.; Tumino, R.; Johansson, G.; Johansson, I.; Ardanaz, E.; Sacerdote, C.; Sonestedt, E.; Ericson, U.; Clavel-Chapelon, F.; Boutron-Ruault, M.-C.; Fagherazzi, G.; Salvini, S.; Amiano, P.; Riboli, E.; González, C. A. Estimation of the intake of

anthocyanidins and their food sources in the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *The British Journal of Nutrition*, **2011**, *106* (7), 1090–1099.

15. Ovaskainen, M.-L.; Törrönen, R.; Koponen, J. M.; Sinkko, H.; Hellström, J.; Reinivuo, H.; Mattila, P. Dietary intake and major food sources of polyphenols in Finnish adults. *The Journal of Nutrition*, **2008**, *138* (3), 562–566.

16. Igwe, E. O.; Charlton, K. E.; Probst, Y. C. Usual dietary anthocyanin intake, sources and their association with blood pressure in a representative sample of Australian adults. *Journal of Human Nutrition and Dietetics*, **2019**, *32* (5), 578–590.

17. Murphy, K. J.; Walker, K. M.; Dyer, K. A.; Bryan, J. Estimation of daily intake of flavonoids and major food sources in middle-aged Australian men and women. *Nutrition Research*, **2019**, *61*, 64–81.

18. Sebastian, R. S.; Wilkinson Enns, C.; Goldman, J. D.; Martin, C. L.; Steinfeldt, L. C.; Murayi, T.; Moshfegh, A. J. A new database facilitates characterization of flavonoid intake, sources, and positive associations with diet quality among US adults. *The Journal of Nutrition*, **2015**, *145* (6), 1239–1248.

19. Drossard, C.; Bolzenius, K.; Kunz, C.; Kersting, M. Anthocyanins in the diet of children and adolescents: intake, sources and trends. *European Journal of Nutrition*, **2013**, *52* (2), 667–676.

20. Wallace, T. C.; Giusti, M. M. Anthocyanins. *Advances in Nutrition*, **2015**, *6* (5), 620–622.

21. Cevallos-Casals, B. A.; Cisneros-Zevallos, L. Stability of anthocyanin-based aqueous extracts of Andean purple corn and red-fleshed sweet potato compared to synthetic and natural colorants. *Food Chemistry*, **2004**, *86* (1), 69–77.

22. Stintzing, F. C.; Stintzing, A. S.; Carle, R.; Frei, B.; Wrolstad, R. E. Color and antioxidant properties of cyanidin-based anthocyanin pigments. *Journal of Agricultural and Food Chemistry*, **2002**, *50* (21), 6172–6181.

23. Giusti, M. M.; Wrolstad, R. E. Acylated anthocyanins from edible sources and their applications in food systems. *Biochemical Engineering Journal*, **2003**, *14* (3), 217–225.

24. Andersen, Ø. M.; Jordheim, M. Anthocyanins. In: Encyclopedia of Life Sciences (ELS). John Wiley & Sons, Ltd: Chichester. In *eLS*; American Cancer Society, 2010.

25. Fang, J. Bioavailability of anthocyanins. *Drug Metabolism Reviews*, **2014**, *46* (4), 508–520.

26. Kay, C. D. Aspects of anthocyanin absorption, metabolism and pharmacokinetics in humans. *Nutrition Research Reviews*, **2006**, *19* (01), 137–146.

27. McGhie, T. K.; Walton, M. C. The bioavailability and absorption of anthocyanins: towards a better understanding. *Molecular Nutrition & Food Research*, **2007**, *51* (6), 702–713.

28. Charron, C. S.; Clevidence, B. A.; Britz, S. J.; Novotny, J. A. Effect of dose size on bioavailability of acylated and nonacylated anthocyanins from red cabbage (*Brassica oleracea* L. var. capitata). *J. Agric. Food Chem.*, **2007**, 5354–5362.

29. Charron, C. S.; Kurilich, A. C.; Clevidence, B. A.; Simon, P. W.; Harrison, D. J.; Britz, S. J.; Baer, D. J.; Novotny, J. A. Bioavailability of anthocyanins from purple carrot juice: effects of acylation and plant matrix. *Journal of Agricultural and Food Chemistry*, **2009**, *57* (4), 1226–1230.

30. Kurilich, A. C.; Clevidence, B. A.; Britz, S. J.; Simon, P. W.; Novotny, J. A. Plasma and urine responses are lower for acylated vs nonacylated anthocyanins from raw and cooked purple carrots. *Journal of Agricultural and Food Chemistry*, **2005**, *53* (16), 6537–6542.

31. Keppler, K.; Humpf, H.-U. Metabolism of anthocyanins and their phenolic degradation products by the intestinal microflora. *Bioorganic & Medicinal Chemistry*, **2005**, *13* (17), 5195–5205.

32. Nurmi, T.; Mursu, J.; Heinonen, M.; Nurmi, A.; Hiltunen, R.; Voutilainen, S.

Metabolism of berry anthocyanins to phenolic acids in humans. *Journal of Agricultural and Food Chemistry*, **2009**, *57* (6), 2274–2281.

33. Xie, L.; Lee, S. G.; Vance, T. M.; Wang, Y.; Kim, B.; Lee, J.-Y.; Chun, O. K.; Bolling, B. W. Bioavailability of anthocyanins and colonic polyphenol metabolites following consumption of aronia berry extract. *Food Chemistry*, **2016**, *211*, 860–868.

34. Mueller, D.; Jung, K.; Winter, M.; Rogoll, D.; Melcher, R.; Richling, E. Human intervention study to investigate the intestinal accessibility and bioavailability of anthocyanins from bilberries. *Food Chemistry*, **2017**, *231*, 275–286.

35. Feliciano, R. P.; Boeres, A.; Massacessi, L.; Istas, G.; Ventura, M. R.; Nunes dos Santos, C.; Heiss, C.; Rodriguez-Mateos, A. Identification and quantification of novel cranberry-derived plasma and urinary (poly)phenols. *Archives of Biochemistry and Biophysics*, **2016**, *599*, 31–41.

36. Feliciano, R. P.; Istas, G.; Heiss, C.; Rodriguez-Mateos, A. Plasma and urinary phenolic profiles after acute and repetitive intake of wild blueberry. *Molecules (Basel, Switzerland)*, **2016**, *21* (9), 1120.

37. Zhang, X.; Sandhu, A.; Edirisinghe, I.; Burton-Freeman, B. An exploratory study of red raspberry (*Rubus idaeus* L.) (poly)phenols/metabolites in human biological samples. *Food & Function*, **2018**, *9* (2), 806–818.

38. Iswaldi, I.; Arráez-Román, D.; Gómez-Caravaca, A. M.; Contreras, M. del M.; Uberos, J.; Segura-Carretero, A.; Fernández-Gutiérrez, A. Identification of polyphenols and their metabolites in human urine after cranberry-syrup consumption. *Food and Chemical Toxicology*, **2013**, *55*, 484–492.

39. Kalt, W. Anthocyanins and their C6-C3-C6 metabolites in humans and animals. *Molecules*, **2019**, *24* (22), 4024.

40. de Ferrars, R. M.; Cassidy, A.; Curtis, P.; Kay, C. D. Phenolic metabolites of anthocyanins following a dietary intervention study in post-menopausal women. *Molecular Nutrition & Food Research*, **2014**, *58* (3), 490–502.

41. Fossen, T.; Andersen, Ø. M. Anthocyanins from tubers and shoots of the purple potato, *Solanum tuberosum*. *Journal of Horticultural Science and Biotechnology*, **2000**, *75* (3), 360–363.

42. Ek, K. L.; Brand-Miller, J.; Copeland, L. Glycemic effect of potatoes. *Food Chemistry*, **2012**, *133* (4), 1230–1240.

43. Fernandes, G.; Velangi, A.; Wolever, T. M. S. Glycemic index of potatoes commonly consumed in North America. *Journal of the American Dietetic Association*, **2005**, *105* (4), 557–562.

44. Burlingame, B.; Mouillé, B.; Charrondière, R. Nutrients, bioactive non-nutrients and anti-nutrients in potatoes. *Journal of Food Composition and Analysis*, **2009**, *22* (6), 494–502.

45. Lachman, J.; Hamouz, K.; Šulc, M.; Orsák, M.; Pivec, V.; Hejtmánková, A.; Dvořák, P.; Čepl, J. Cultivar differences of total anthocyanins and anthocyanidins in red and purple-fleshed potatoes and their relation to antioxidant activity. *Food Chemistry*, **2009**, *114* (3), 836–843.

46. Mori, M.; Hayashi, K.; Ohara-Takada, A.; Watanuki, H.; Katahira, R.; Ono, H.; Terahara, N. Anthocyanins from skins and fleshes of potato varieties. *Food Science and Technology Research*, **2010**, *16* (2), 115–122.

47. Andre, C. M.; Ghislain, M.; Bertin, P.; Oufir, M.; del Rosario Herrera, M.; Hoffmann, L.; Hausman, J.-F.; Larondelle, Y.; Evers, D. Andean potato cultivars (*Solanum tuberosum* L.) as a source of antioxidant and mineral micronutrients. *Journal of Agricultural and Food Chemistry*, **2007**, *55* (2), 366–378.

48. Brown, C. R.; Wrolstad, R.; Durst, R.; Yang, C.-P.; Clevidence, B. Breeding studies in potatoes containing high concentrations of anthocyanins. *American Journal of Potato Research*, **2003**, *80* (4), 241–249.

49. Reyes, L. F.; Cisneros-Zevallos, L. Degradation kinetics and colour of anthocyanins in aqueous extracts of purple-

and red-flesh potatoes (*Solanum tuberosum* L.). *Food Chemistry*, **2007**, *100* (3), 885–894.

50. Hess, T.; Chatterton, J.; Daccache, A.; Williams, A. The impact of changing food choices on the blue water scarcity footprint and greenhouse gas emissions of the British diet: the example of potato, pasta and rice. *Journal of Cleaner Production*, **2016**, *112*, 4558–4568.

51. Huamán, Z. *Systematic Botany and Morphology of the Potato*; International Potato Center, 1980.

52. Spooner, D. M.; McLean, K.; Ramsay, G.; Waugh, R.; Bryan, G. J. A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceedings of the National Academy of Sciences*, **2005**, *102* (41), 14694–14699.

53. Spooner, D. M.; Ghislain, M.; Simon, R.; Jansky, S. H.; Gavrilenko, T. Systematics, diversity, genetics, and evolution of wild and cultivated potatoes. *The Botanical Review*, **2014**, *80* (4), 283–383.

54. van Eck, H. J. Genetics of morphological and tuber traits. In *Potato Biology and Biotechnology*; Vreugdenhil, D., Bradshaw, J., Gebhardt, C., Govers, F., Mackerron, D. K. L., Taylor, M. A., Ross, H. A. (Editors); Elsevier Science B.V., Amsterdam, 2007; pp. 91–115.

55. Jansen, G.; Flamme, W. Coloured potatoes (*Solanum tuberosum* L.) – anthocyanin content and tuber quality. *Genetic Resources and Crop Evolution*, **2006**, *53* (7), 1321.

56. Hejtmánková, K.; Kotíková, Z.; Hamouz, K.; Pivec, V.; Vacek, J.; Lachman, J. Influence of flesh colour, year and growing area on carotenoid and anthocyanin content in potato tubers. *Journal of Food Composition and Analysis*, **2013**, *32* (1), 20–27.

57. Ieri, F.; Innocenti, M.; Andrenelli, L.; Vecchio, V.; Mulinacci, N. Rapid HPLC/DAD/MS method to determine phenolic acids, glycoalkaloids and anthocyanins in pigmented potatoes (*Solanum tuberosum* L.) and correlations with variety and geographical origin. *Food Chemistry*, **2011**, *125* (2), 750–759.

58. Oertel, A.; Matros, A.; Hartmann, A.; Arapitsas, P.; Dehmer, K. J.; Martens, S.; Mock, H.-P. Metabolite profiling of red and blue potatoes revealed cultivar and tissue specific patterns for anthocyanins and other polyphenols. *Planta*, **2017**, *246* (2), 281–297.

59. Reyes, L. F.; Miller, J. C.; Cisneros-Zevallos, L. Environmental conditions influence the content and yield of anthocyanins and total phenolics in purple- and red-flesh potatoes during tuber development. *American Journal of Potato Research*, **2004**, *81* (3), 187.

60. Liu, Y.; Tikunov, Y.; Schouten, R. E.; Marcelis, L. F. M.; Visser, R. G. F.; Bovy, A. Anthocyanin biosynthesis and degradation mechanisms in Solanaceous vegetables: a review. *Frontiers in Chemistry*, **2018**, *6*, 52.

61. Wink, M. Plant breeding: importance of plant secondary metabolites for protection against pathogens and herbivores. *Theoretical and Applied Genetics*, **1988**, *75* (2), 225–233.

62. Payyavula, R. S.; Navarre, D. A.; Kuhl, J. C.; Pantoja, A.; Pillai, S. S. Differential effects of environment on potato phenylpropanoid and carotenoid expression. *BMC Plant Biology*, **2012**, *12*, 39.

63. Kong, J.-M.; Chia, L.-S.; Goh, N.-K.; Chia, T.-F.; Brouillard, R. Analysis and biological activities of anthocyanins. *Phytochemistry*, **2003**, *64* (5), 923–933.

64. Clifford, M. N. Anthocyanins – nature, occurrence and dietary burden. *Journal of the Science of Food and Agriculture*, **2000**, *80* (7), 1063–1072.

65. Andersen, Ø. M.; Opheim, S.; Aksnes, D. W.; Frøystein, N. Å. Structure of petanin, an acylated anthocyanin isolated from *Solanum tuberosum*, using homo-and hetero-nuclear two-dimensional nuclear magnetic resonance techniques. *Phytochemical Analysis*, **1991**, *2* (5), 230–236.

66. Eichhorn, S.; Winterhalter, P. Anthocyanins from pigmented potato (*Solanum tuberosum* L.) varieties. *Food Research International*, **2005**, *38* (8–9), 943–948.

67. Fossen, T.; Øvstedal, D. O.; Slimestad, R.; Andersen, Ø. M. Anthocyanins from a Norwegian potato cultivar. *Food Chemistry*, **2003**, *81* (3), 433–437.

68. Giusti, M. M.; Polit, M. F.; Ayvaz, H.; Tay, D.; Manrique, I. Characterization and quantitation of anthocyanins and other phenolics in native Andean potatoes. *Journal of Agricultural and Food Chemistry*, **2014**, 4408–4016.

69. Harborne, J. B. Plant polyphenols. I. Anthocyanin production in the cultivated potato. *The Biochemical Journal*, **1960**, *74*, 262–269.

70. Hillebrand, S.; Naumann, H.; Kitzinski, N.; Köhler, N.; Winterhalter, P. Isolation and characterization of anthocyanins from blue-fleshed potatoes (*Solanum tuberosum* L.). In *Food 3 (Special issue 1)*; Global Science Books; 2009; pp. 96–101.

71. Howard, H. W.; Kukimura, H.; Whitmore, E. T. The anthocyanin pigments of the tubers and sprouts of *Tuberosum* potatoes. *Potato Research*, **1970**, *13* (2), 142–145.

72. Mulinacci, N.; Ieri, F.; Giaccherini, C.; Innocenti, M.; Andrenelli, L.; Canova, G.; Saracchi, M.; Casiraghi, M. C. Effect of cooking on the anthocyanins, phenolic acids, glycoalkaloids, and resistant starch content in two pigmented cultivars of *Solanum tuberosum* L. *Journal of Agricultural and Food Chemistry*, **2008**, *56* (24), 11830–11837.

73. Naito, K.; Umemura, Y.; Mori, M.; Sumida, T.; Okada, T.; Takamatsu, N.; Okawa, Y.; Hayashi, K.; Saito, N.; Honda, T. Acylated pelargonidin glycosides from a red potato. *Phytochemistry*, **1998**, *47* (1), 109–112.

74. Pillai, S. S. Analysis of polyphenols, anthocyanins and carotenoids in tubers from *Solanum tuberosum* group Phureja, Stenotomum and Andigena. *American Journal of Potato Research*, **2013**, *90* (5), 440.

75. Rodriguez-Saona, L. E.; Giusti, M. M.; Wrolstad, R. E. Anthocyanin pigment composition of red-fleshed potatoes. *Journal of Food Science*, **1998**, *63* (3), 458–465.

76. Mazza, G.; Brouillard, R. Recent developments in the stabilization of anthocyanins in food products. *Food Chemistry*, **1987**, *25* (3), 207–225.

77. Borkowski, T.; Szymusiak, H.; Gliszczyńska-Swigło, A.; Tyrakowska, B. The effect of 3-*O*-β-glucosylation on structural transformations of anthocyanidins. *Food Research International*, **2005**, *38* (8–9), 1031–1037.

78. de Pascual-Teresa, S.; Sanchez-Ballesta, M. T. Anthocyanins: from plant to health. *Phytochemistry Reviews*, **2007**, *7* (2), 281–299.

79. Tatsuzawa, F.; Saito, N.; Miyoshi, K.; Shinoda, K.; Shigihara, A.; Honda, T. Diacylated 8-C-glucosylcyanidin 3-glucoside from the flowers of *Tricyrtis formosana*. *Chemical & Pharmaceutical Bulletin*, **2004**, *52* (5), 631–633.

80. Harborne, J. B.; Williams, C. A. Anthocyanins and other flavonoids. *Natural Product Reports*, **1998**, *15* (6), 631.

81. Harborne, J. B. Chromatographic identification of anthocyanin pigments. *Chromatographic Reviews*, **1959**, *1*, 209–224.

82. Dodds, K. S.; Long, D. H. The inheritance of colour in diploid potatoes I. Types of anthocyanidins and their genetic loci. *Journal of Genetics*, **1955**, *53* (1), 136–149.

83. Tengkun, N.; Dongdong, W.; Xiaohui, M.; Yue, C.; Qin, C. Analysis of key genes involved in potato anthocyanin biosynthesis based on genomics and transcriptomics data. *Frontiers in Plant Science*, **2019**, *10*, 603.

84. Hung, C. Y.; Murray, J. R.; Ohmann, S. M.; Tong, C. B. S. Anthocyanin accumulation during potato tuber development. *American Society for Horticultural Science (USA)*, **1997**, *122* (1), 20–23.

85. Azuma, K.; Ohyama, A.; Ippoushi, K.; Ichiyanagi, T.; Takeuchi, A.; Saito, T.;

Fukuoka, H. Structures and antioxidant activity of anthocyanins in many accessions of eggplant and its related species. *Journal of Agricultural and Food Chemistry*, **2008**, *56* (21), 10154–10159.

86. Butelli, E.; Titta, L.; Giorgio, M.; Mock, H.-P.; Matros, A.; Peterek, S.; Schijlen, E. G. W. M.; Hall, R. D.; Bovy, A. G.; Luo, J.; Martin, C. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nature Biotechnology*, **2008**, *26* (11), 1301–1308.

87. Sadilova, E.; Stintzing, F. C.; Carle, R. Anthocyanins, colour and antioxidant properties of eggplant (*Solanum melongena* L.) and violet pepper (*Capsicum annuum* L.) peel extracts. *Zeitschrift Fur Naturforschung. C, Journal of Biosciences*, **2006**, *61* (7–8), 527–535.

88. Odake, K.; Terahara, N.; Saito, N.; Toki, K.; Honda, T. Chemical structures of two anthocyanins from purple sweet potato, Ipomoea batatas. *Phytochemistry*, **1992**, *31* (6), 2127–2130.

89. Wu, X.; Prior, R. L. Systematic identification and characterization of anthocyanins by HPLC-ESI-MS/MS in common foods in the United States: fruits and berries. *Journal of Agricultural and Food Chemistry*, **2005**, *53* (7), 2589–2599.

90. Stalmach, A.; Edwards, C. A.; Wightman, J. D.; Crozier, A. Identification of (poly)phenolic compounds in Concord grape juice and their metabolites in human plasma and urine after juice consumption. *Journal of Agricultural and Food Chemistry*, **2011**, *59* (17), 9512–9522.

91. Cebadera-Miranda, L.; Domínguez, L.; Dias, M. I.; Barros, L.; Ferreira, I. C. F. R.; Igual, M.; Martínez-Navarrete, N.; Fernández-Ruiz, V.; Morales, P.; Cámara, M. Sanguinello and tarocco (*Citrus sinensis* L. Osbeck): bioactive compounds and colour appearance of blood oranges. *Food Chemistry*, **2019**, *270*, 395–402.

92. Kidøy, L.; Nygård, A. M.; Andersen, Ø. M.; Pedersen, A. T.; Aksnes, D. W.; Kiremire, B. T. Anthocyanins in fruits of *Passiflora edulis* and *P. suberosa*. *Journal of Food Composition and Analysis*, **1997**, *10* (1), 49–54.

93. Moreno, D. A.; Pérez-Balibrea, S.; Ferreres, F.; Gil-Izquierdo, Á.; García-Viguera, C. Acylated anthocyanins in broccoli sprouts. *Food Chemistry*, **2010**, *123* (2), 358–363.

94. Lao, F.; Giusti, M. M. Quantification of purple corn (*Zea mays* L.) anthocyanins using spectrophotometric and HPLC approaches: method comparison and correlation. *Food Analytical Methods*, **2016**, *9* (5), 1367–1380.

95. Xu, J.; Su, X.; Lim, S.; Griffin, J.; Carey, E.; Katz, B.; Tomich, J.; Smith, J. S.; Wang, W. Characterisation and stability of anthocyanins in purple-fleshed sweet potato P40. *Food Chemistry*, **2015**, *186*, 90–96.

96. Cheng, D. M.; Pogrebnyak, N.; Kuhn, P.; Poulev, A.; Waterman, C.; Rojas-Silva, P.; Johnson, W. D.; Raskin, I. Polyphenol-rich Rutgers Scarlet lettuce improves glucose metabolism and liver lipid accumulation in diet-induced obese C57BL/6 mice. *Nutrition*, **2014**, *30* (7–8), S52-58.

97. Cabrita, L.; Fossen, T.; Andersen, Ø. M. Colour and stability of the six common anthocyanidin 3-glucosides in aqueous solutions. *Food Chemistry*, **2000**, *68* (1), 101–107.

98. Davies, A. J.; Mazza, G. Copigmentation of simple and acylated anthocyanins with colorless phenolic compounds. *Journal of Agricultural and Food Chemistry*, **1993**, *41* (5), 716–720.

99. Sondheimer, E.; Kertesz, Z. I. Participation of ascorbic acid in the destruction of anthocyanin in strawberry juice and model systems. *Journal of Food Science*, **1953**, *18* (1–6), 475–479.

100. Dangles, O.; Fenger, J.-A. The chemical reactivity of anthocyanins and its consequences in food science and nutrition. *Molecules : A Journal of Synthetic Chemistry and Natural Product Chemistry*, **2018**, *23* (8), 1970.

101. Pina, F.; Melo, M. J.; Laia, C. A. T.; Parola, A. J.; Lima, J. C. Chemistry and applications of flavylium compounds: a

handful of colours. *Chemical Society Reviews*, **2012**, *41* (2), 869–908.

102. Brouillard, R.; Dubois, J.-E. Mechanism of the structural transformations of anthocyanins in acidic media. *Journal of the American Chemical Society*, **1977**, *99* (5), 1359–1364.

103. Brouillard, R.; Delaporte, B. Chemistry of anthocyanin pigments. 2. Kinetic and thermodynamic study of proton transfer, hydration, and tautomeric reactions of malvidin 3-glucoside. *Journal of the American Chemical Society*, **1977**, *99* (26), 8461–8468.

104. Brouillard, R.; Lang, J. The hemiacetal–cis-chalcone equilibrium of malvin, a natural anthocyanin. *Canadian Journal of Chemistry*, **1990**, *68* (5), 755–761.

105. Santos, H.; Turner, D. L.; Lima, J. C.; Figueiredo, P.; Pina, F. S.; Maçanita, A. L. Elucidation of the multiple equilibria of malvin in aqueous solution by one- and two-dimensional NMR. *Phytochemistry*, **1993**, *33* (5), 1227–1232.

106. Roblegg, E.; Coughran, A.; Sirjani, D. Saliva: an all-rounder of our body. *European Journal of Pharmaceutics and Biopharmaceutics*, **2019**, *142*, 133–141.

107. Ovesen, L.; Bendtsen, F.; Tage-Jensen, U.; Pedersen, N. T.; Gram, B. R.; Rune, S. J. Intraluminal pH in the stomach, duodenum, and proximal jejunum in normal subjects and patients with exocrine pancreatic insufficiency. *Gastroenterology*, **1986**, *90* (4), 958–962.

108. Dressman, J. B.; Berardi, R. R.; Dermentzoglou, L. C.; Russell, T. L.; Schmaltz, S. P.; Barnett, J. L.; Järvenpää, K. M. Upper gastrointestinal (GI) pH in young, healthy men and women. *Pharmaceutical Research*, **1990**, *7* (7), 756–761.

109. Fleschhut, J.; Kratzer, F.; Rechkemmer, G.; Kulling, S. E. Stability and biotransformation of various dietary anthocyanins in vitro. *European Journal of Nutrition*, **2006**, *45* (1), 7–18.

110. Castañeda-Ovando, A.; Pacheco-Hernández, Ma. de L.; Páez-Hernández, Ma. E.; Rodríguez, J. A.; Galán-Vidal, C. A. Chemical studies of anthocyanins: a review. *Food Chemistry*, **2009**, *113* (4), 859–871.

111. Fossen, T.; Cabrita, L.; Andersen, O. M. Colour and stability of pure anthocyanins influenced by pH including the alkaline region. *Food Chemistry*, **1998**, *63* (4), 435–440.

112. Yoshida, K.; Kondo, T.; Goto, T. Intramolecular stacking conformation of gentiodelphin, a diacylated anthocyanin from *Gentiana makinoi*. *Tetrahedron*, **1992**, *48* (21), 4313–4326.

113. Dangles, O.; Saito, N.; Brouillard, R. Anthocyanin intramolecular copigment effect. *Phytochemistry*, **1993**, *34* (1), 119–124.

114. Nerdal, W.; Andersen, Ø. M. Evidence for self-association of the anthocyanin petanin in acidified, methanolic solution using two-dimensional nuclear overhauser enhancement NMR experiments and distance geometry calculations. *Phytochemical Analysis*, **1991**, *2* (6), 263–270.

115. Brouillard, R. Origin of the exceptional colour stability of the Zebrina anthocyanin. *Phytochemistry*, **1981**, *20* (1), 143–145.

116. Nerdal, W.; Andersen, Ø. M. Intermolecular aromatic acid association of an anthocyanin (petanin) evidenced by two-dimensional nuclear overhauser enhancement nuclear magnetic resonance experiments and distance geometry calculations. *Phytochemical Analysis*, **1992**, *3* (4), 182–189.

117. Cardona, F.; Andrés-Lacueva, C.; Tulipani, S.; Tinahones, F. J.; Queipo-Ortuño, M. I. Benefits of polyphenols on gut microbiota and implications in human health. *The Journal of Nutritional Biochemistry*, **2013**, *24* (8), 1415–1422.

118. Mallery, S. R.; Budendorf, D. E.; Larsen, M. P.; Pei, P.; Tong, M.; Holpuch, A. S.; Larsen, P. E.; Stoner, G. D.; Fields, H. W.; Chan, K. K.; Ling, Y.; Liu, Z. Effects of human oral mucosal tissue, saliva, and oral microflora on intraoral metabolism and bioactivation of black raspberry anthocyanins. *Cancer Prevention Research (Philadelphia, Pa.)*, **2011**, *4* (8), 1209–1221.

119. Kamonpatana, K.; Giusti, M. M.; Chitchumroonchokchai, C.; MorenoCruz, M.; Riedl, K. M.; Kumar, P.; Failla, M. L. Susceptibility of anthocyanins to *ex vivo* degradation in human saliva. *Food Chemistry*, **2012**, *135* (2), 738–747.

120. Kamonpatana, K.; Failla, M. L.; Kumar, P. S.; Giusti, M. M. Anthocyanin structure determines susceptibility to microbial degradation and bioavailability to the buccal mucosa. *Journal of Agricultural and Food Chemistry*, **2014**, *62* (29), 6903–6910.

121. Oliveira, H.; Perez-Gregório, R.; de Freitas, V.; Mateus, N.; Fernandes, I. Comparison of the *in vitro* gastrointestinal bioavailability of acylated and non-acylated anthocyanins: purple-fleshed sweet potato vs red wine. *Food Chemistry*, **2019**, *276*, 410–418.

122. Cao, G.; Prior, R. L. Anthocyanins are detected in human plasma after oral administration of an elderberry extract. *Clinical Chemistry*, **1999**, *45* (4), 574–576.

123. Cao, G.; Muccitelli, H. U.; Sánchez-Moreno, C.; Prior, R. L. Anthocyanins are absorbed in glycated forms in elderly women: a pharmacokinetic study. *The American Journal of Clinical Nutrition*, **2001**, *73* (5), 920–926.

124. Milbury, P. E.; Cao, G.; Prior, R. L.; Blumberg, J. Bioavailablility of elderberry anthocyanins. *Mechanisms of Ageing and Development*, **2002**, *123* (8), 997–1006.

125. Tsuda, T.; Horio, F.; Osawa, T. Absorption and metabolism of cyanidin 3-*O*-beta-D-glucoside in rats. *FEBS letters*, **1999**, *449* (2–3), 179–182.

126. Felgines, C.; Talavéra, S.; Gonthier, M.-P.; Texier, O.; Scalbert, A.; Lamaison, J.-L.; Rémésy, C. Strawberry anthocyanins are recovered in urine as glucuro- and sulfoconjugates in humans. *The Journal of Nutrition*, **2003**, *133* (5), 1296–1301.

127. Passamonti, S.; Vrhovsek, U.; Vanzo, A.; Mattivi, F. The stomach as a site for anthocyanins absorption from food. *FEBS Letters*, **2003**, *544* (1–3), 210–213.

128. Talavéra, S.; Felgines, C.; Texier, O.; Besson, C.; Lamaison, J.-L.; Rémésy, C. Anthocyanins are efficiently absorbed from the stomach in anesthetized rats. *The Journal of Nutrition*, **2003**, *133* (12), 4178–4182.

129. Talavéra, S.; Felgines, C.; Texier, O.; Besson, C.; Gil-Izquierdo, A.; Lamaison, J.-L.; Rémésy, C. Anthocyanin metabolism in rats and their distribution to digestive area, kidney, and brain. *Journal of Agricultural and Food Chemistry*, **2005**, *53* (10), 3902–3908.

130. Passamonti, S.; Vrhovsek, U.; Mattivi, F. The interaction of anthocyanins with bilitranslocase. *Biochemical and Biophysical Research Communications*, **2002**, *296* (3), 631–636.

131. Oliveira, H.; Fernandes, I.; Brás, N. F.; Faria, A.; De Freitas, V.; Calhau, C.; Mateus, N. Experimental and theoretical data on the mechanism by which red wine anthocyanins are transported through a human MKN-28 gastric cell model. *Journal of Agricultural and Food Chemistry*, **2015**, *63* (35), 7685–7692.

132. Oliveira, H.; Roma-Rodrigues, C.; Santos, A.; Veigas, B.; Brás, N.; Faria, A.; Calhau, C.; de Freitas, V.; Baptista, P. V.; Mateus, N.; Fernandes, A. R.; Fernandes, I. GLUT1 and GLUT3 involvement in anthocyanin gastric transport - nanobased targeted approach. *Scientific Reports*, **2019**, *9* (1), 789.

133. Kubow, S.; Iskandar, M. M.; Melgar-Bermudez, E.; Sleno, L.; Sabally, K.; Azadi, B.; How, E.; Prakash, S.; Burgos, G.; Felde, T. Z. Effects of simulated human gastrointestinal digestion of two purple-fleshed potato cultivars on anthocyanin composition and cytotoxicity in colonic cancer and non-tumorigenic cells. *Nutrients*, **2017**, *9* (9), 953.

134. Kubow, S.; Iskandar, M. M.; Sabally, K.; Azadi, B.; Sadeghi Ekbatan, S.; Kumarathasan, P.; Das, D. D.; Prakash, S.; Burgos, G.; Zum Felde, T. Biotransformation of anthocyanins from two purple-fleshed sweet potato accessions in a dynamic gastrointestinal system. *Food Chemistry*, **2016**, *192*, 171–177.

135. Podsędek, A.; Redzynia, M.; Klewicka, E.; Koziołkiewicz, M. Matrix effects on the

stability and antioxidant activity of red cabbage anthocyanins under simulated gastrointestinal digestion. *BioMed Research International*, **2014**, *2014*, 365738.

136. Ichiyanagi, T.; Terahara, N.; Rahman, M. M.; Konishi, T. Gastrointestinal uptake of nasunin, acylated anthocyanin in eggplant. *Journal of Agricultural and Food Chemistry*, **2006**, *54* (15), 5306–5312.

137. Han, F.; Oliveira, H.; Brás, N. F.; Fernandes, I.; Cruz, L.; De Freitas, V.; Mateus, N. *In vitro* gastrointestinal absorption of red wine anthocyanins – impact of structural complexity and phase II metabolization. *Food Chemistry*, **2020**, *317*, 126398.

138. Felgines, C.; Talavéra, S.; Texier, O.; Besson, C.; Fogliano, V.; Lamaison, J.-L.; la Fauci, L.; Galvano, G.; Rémésy, C.; Galvano, F. Absorption and metabolism of red orange juice anthocyanins in rats. *The British Journal of Nutrition*, **2006**, *95* (5), 898–904.

139. Suda, I.; Oki, T.; Masuda, M.; Nishiba, Y.; Furuta, S.; Matsugano, K.; Sugita, K.; Terahara, N. Direct absorption of acylated anthocyanin in purple-fleshed sweet potato into rats. *Journal of Agricultural and Food Chemistry*, **2002**, *50* (6), 1672–1676.

140. Harada, K.; Kano, M.; Takayanagi, T.; Yamakawa, O.; Ishikawa, F. Absorption of acylated anthocyanins in rats and humans after ingesting an extract of *Ipomoea batatas* purple sweet potato tuber. *Bioscience, Biotechnology, and Biochemistry*, **2004**, *68* (7), 1500–1507.

141. Talavéra, S.; Felgines, C.; Texier, O.; Besson, C.; Manach, C.; Lamaison, J.-L.; Rémésy, C. Anthocyanins are efficiently absorbed from the small intestine in rats. *The Journal of Nutrition*, **2004**, *134* (9), 2275–2279.

142. Hassimotto, N. M. A.; Genovese, M. I.; Lajolo, F. M. Absorption and metabolism of cyanidin-3-glucoside and cyanidin-3-rutinoside extracted from wild mulberry (*Morus nigra* L.) in rats. *Nutrition Research*, **2008**, *28* (3), 198–207.

143. Matuschek, M. C.; Hendriks, W. H.; McGhie, T. K.; Reynolds, G. W. The jejunum is the main site of absorption for anthocyanins in mice. *The Journal of Nutritional Biochemistry*, **2006**, *17* (1), 31–36.

144. Faria, A.; Pestana, D.; Azevedo, J.; Martel, F.; de Freitas, V.; Azevedo, I.; Mateus, N.; Calhau, C. Absorption of anthocyanins through intestinal epithelial cells – putative involvement of GLUT2. *Molecular Nutrition & Food Research*, **2009**, *53* (11), 1430–1437.

145. Zou, T.-B.; Feng, D.; Song, G.; Li, H.-W.; Tang, H.-W.; Ling, W.-H. The role of sodium-dependent glucose transporter 1 and glucose transporter 2 in the absorption of cyanidin-3-*O*-β-glucoside in Caco-2 cells. *Nutrients*, **2014**, *6* (10), 4165–4177.

146. Mülleder, U.; Murkovic, M.; Pfannhauser, W. Urinary excretion of cyanidin glycosides. *Journal of Biochemical and Biophysical Methods*, **2002**, *53* (1–3), 61–66.

147. Zhang, H.; Hassan, Y. I.; Renaud, J.; Liu, R.; Yang, C.; Sun, Y.; Tsao, R. Bioaccessibility, bioavailability, and anti-inflammatory effects of anthocyanins from purple root vegetables using mono- and co-culture cell models. *Molecular Nutrition & Food Research*, **2017**, *61* (10), 1600928.

148. Yi, W.; Akoh, C. C.; Fischer, J.; Krewer, G. Absorption of anthocyanins from blueberry extracts by Caco-2 human intestinal cell monolayers. *Journal of Agricultural and Food Chemistry*, **2006**, *54* (15), 5651–5658.

149. Novotny, J. A.; Clevidence, B. A.; Kurilich, A. C. Anthocyanin kinetics are dependent on anthocyanin structure. *The British Journal of Nutrition*, **2012**, *107* (4), 504–509.

150. Aura, A.-M.; Martin-Lopez, P.; O'Leary, K. A.; Williamson, G.; Oksman-Caldentey, K.-M.; Poutanen, K.; Santos-Buelga, C. *In vitro* metabolism of anthocyanins by human gut microflora. *European Journal of Nutrition*, **2005**, *44* (3), 133–142.

151. González-Barrio, R.; Edwards, C. A.; Crozier, A. Colonic catabolism of ellagitannins, ellagic acid, and raspberry anthocyanins: *in vivo* and *in vitro* studies.

Drug Metabolism and Disposition, **2011**, *39* (9), 1680–1688.

152. Hidalgo, M.; Oruna-Concha, M. J.; Kolida, S.; Walton, G. E.; Kallithraka, S.; Spencer, J. P. E.; de Pascual-Teresa, S. Metabolism of anthocyanins by human gut microflora and their influence on gut bacterial growth. *Journal of Agricultural and Food Chemistry*, **2012**, *60* (15), 3882–3890.

153. Haughton, E.; Clifford, M. N.; Sharp, P. Monocarboxylate transporter expression is associated with the absorption of benzoic acid in human intestinal epithelial cells. *Journal of the Science of Food and Agriculture*, **2007**, *87* (2), 239–244.

154. Overall, J.; Bonney, S. A.; Wilson, M.; Beermann, A.; Grace, M. H.; Esposito, D.; Lila, M. A.; Komarnytsky, S. Metabolic effects of berries with structurally diverse anthocyanins. *International Journal of Molecular Sciences*, **2017**, *18* (2), 422.

155. Stalmach, A.; Edwards, C. A.; Wightman, J. D.; Crozier, A. Colonic catabolism of dietary phenolic and polyphenolic compounds from Concord grape juice. *Food & Function*, **2013**, *4* (1), 52–62.

156. Kalt, W.; Liu, Y.; McDonald, J. E.; Vinqvist-Tymchuk, M. R.; Fillmore, S. A. E. Anthocyanin metabolites are abundant and persistent in human urine. *Journal of Agricultural and Food Chemistry*, **2014**, *62*, 3926–3934.

157. Wu, B.; Kulkarni, K.; Basu, S.; Zhang, S.; Hu, M. First-pass metabolism via UDP-glucuronosyltransferase: a barrier to oral bioavailability of phenolics. *Journal of Pharmaceutical Sciences*, **2011**, *100* (9), 3655–3681.

158. de Ferrars, R. M.; Czank, C.; Zhang, Q.; Botting, N. P.; Kroon, P. A.; Cassidy, A.; Kay, C. D. The pharmacokinetics of anthocyanins and their metabolites in humans: pharmacokinetics of a ^{13}C-labelled anthocyanin. *British Journal of Pharmacology*, **2014**, *171* (13), 3268–3282.

159. Czank, C.; Cassidy, A.; Zhang, Q.; Morrison, D. J.; Preston, T.; Kroon, P. A.; Botting, N. P.; Kay, C. D. Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a ^{13}C-tracer study. *The American Journal of Clinical Nutrition*, **2013**, *97* (5), 995–1003.

160. Wiczkowski, W.; Szawara-Nowak, D.; Romaszko, J. The impact of red cabbage fermentation on bioavailability of anthocyanins and antioxidant capacity of human plasma. *Food Chemistry*, **2016**, *190*, 730–740.

161. Woodward, G. M.; Needs, P. W.; Kay, C. D. Anthocyanin-derived phenolic acids form glucuronides following simulated gastrointestinal digestion and microsomal glucuronidation. *Molecular Nutrition & Food Research*, **2011**, *55* (3), 378–386.

162. Kay, C. D.; Mazza, G.; Holub, B. J.; Wang, J. Anthocyanin metabolites in human urine and serum. *The British journal of nutrition*, **2004**, *91* (6), 933–942.

163. Roberts, M. S.; Magnusson, B. M.; Burczynski, F. J.; Weiss, M. Enterohepatic circulation: physiological, pharmacokinetic and clinical implications. *Clinical Pharmacokinetics*, **2002**, *41* (10), 751–790.

164. Passamonti, S.; Vanzo, A.; Vrhovsek, U.; Terdoslavich, M.; Cocolo, A.; Decorti, G.; Mattivi, F. Hepatic uptake of grape anthocyanins and the role of bilitranslocase. *Food Research International*, **2005**, *38* (8–9), 953–960.

165. Marczylo, T. H.; Cooke, D.; Brown, K.; Steward, W. P.; Gescher, A. J. Pharmacokinetics and metabolism of the putative cancer chemopreventive agent cyanidin-3-glucoside in mice. *Cancer Chemotherapy and Pharmacology*, **2009**, *64* (6), 1261–1268.

166. Felgines, C.; Krisa, S.; Mauray, A.; Besson, C.; Lamaison, J.-L.; Scalbert, A.; Mérillon, J.-M.; Texier, O. Radiolabelled cyanidin 3-*O*-glucoside is poorly absorbed in the mouse. *The British Journal of Nutrition*, **2010**, *103* (12), 1738–1745.

167. Kay, C. D.; Mazza, G. J.; Holub, B. J. Anthocyanins exist in the circulation primarily as metabolites in adult men. *The Journal of Nutrition*, **2005**, *135* (11), 2582–2588.

168. Matsumoto, H.; Inaba, H.; Kishi, M.; Tominaga, S.; Hirayama, M.; Tsuda, T.

Orally administered delphinidin 3-rutinoside and cyanidin 3-rutinoside are directly absorbed in rats and humans and appear in the blood as theintact forms. *Journal of Agricultural and Food Chemistry*, **2001**, *49* (3), 1546–1551.

169. Kuntz, S.; Rudloff, S.; Asseburg, H.; Borsch, C.; Fröhling, B.; Unger, F.; Dold, S.; Spengler, B.; Römpp, A.; Kunz, C. Uptake and bioavailability of anthocyanins and phenolic acids from grape/blueberry juice and smoothie in vitro and in vivo. *The British Journal of Nutrition*, **2015**, *113* (7), 1044–1055.

170. Mullen, W.; Edwards, C. A.; Serafini, M.; Crozier, A. Bioavailability of pelargonidin-3-O-glucoside and its metabolites in humans following the ingestion of strawberries with and without cream. *Journal of Agricultural and Food Chemistry*, **2008**, *56* (3), 713–719.

171. Banaszewski, K.; Park, E.; Edirisinghe, I.; Cappozzo, J. C.; Burton-Freeman, B. M. A pilot study to investigate bioavailability of strawberry anthocyanins and characterize postprandial plasma polyphenols absorption patterns by Q-TOF LC/MS in humans. *Journal of Berry Research*, **2013**, *3* (2), 113–126.

172. Wu, X.; Cao, G.; Prior, R. L. Absorption and metabolism of anthocyanins in elderly women after consumption of elderberry or blueberry. *The Journal of Nutrition*, **2002**, *132* (7), 1865–1871.

173. Felgines, C.; Talavera, S.; Texier, O.; Gil-Izquierdo, A.; Lamaison, J.-L.; Remesy, C. Blackberry anthocyanins are mainly recovered from urine as methylated and glucuronidated conjugates in humans. *Journal of Agricultural and Food Chemistry*, **2005**, *53* (20), 7721–7727.

174. Carkeet, C.; Clevidence, B. A.; Novotny, J. A. Anthocyanin excretion by humans increases linearly with increasing strawberry dose. *The Journal of Nutrition*, **2008**, *138* (5), 897–902.

175. Tsang, C.; Smail, N. F.; McDougall, G. J.; Almoosawi, S.; Al-Dujaili, E. A. Bioavailability and urinary excretion of phenolic-derived metabolites after acute consumption of purple Majesty potato in humans. *EC Nutrition*, **2015**, *1.3*, 96–105.

176. Oki, T.; Suda, I.; Terahara, N.; Sato, M.; Hatakeyama, M. Determination of acylated anthocyanin in human urine after ingesting a purple-fleshed sweet potato beverage with various contents of anthocyanin by LC-ESI-MS/MS. *Bioscience, biotechnology, and biochemistry*, **2006**, *70* (10), 2540–2543.

177. He, J.; Magnuson, B. A.; Lala, G.; Tian, Q.; Schwartz, S. J.; Giusti, M. M. Intact anthocyanins and metabolites in rat urine and plasma after 3 months of anthocyanin supplementation. *Nutrition and cancer*, **2006**, *54* (1), 3–12.

178. Nielsen, I. L. F.; Dragsted, L. O.; Ravn-Haren, G.; Freese, R.; Rasmussen, S. E. Absorption and excretion of black currant anthocyanins in humans and watanabe heritable hyperlipidemic rabbits. *Journal of Agricultural and Food Chemistry*, **2003**, *51* (9), 2813–2820.

179. Mazza, G.; Kay, C. D.; Cottrell, T.; Holub, B. J. Absorption of anthocyanins from blueberries and serum antioxidant status in human subjects. *Journal of Agricultural and Food Chemistry*, **2002**, *50* (26), 7731–7737.

180. Bitsch, R.; Netzel, M.; Frank, T.; Strass, G.; Bitsch, I. Bioavailability and biokinetics of anthocyanins from red grape juice and red wine. *Journal of Biomedicine and Biotechnology*, **2004**, *2004* (5), 293–298.

181. McGhie, T. K.; Ainge, G. D.; Barnett, L. E.; Cooney, J. M.; Jensen, D. J. Anthocyanin glycosides from berry fruit are absorbed and excreted unmetabolized by both humans and rats. *Journal of Agricultural and Food Chemistry*, **2003**, *51* (16), 4539–4548.

182. Eker, M. E.; Aaby, K.; Budic-Leto, I.; Rimac Brnčić, S.; El, S. N.; Karakaya, S.; Simsek, S.; Manach, C.; Wiczkowski, W.; de Pascual-Teresa, S. A review of factors affecting anthocyanin bioavailability: possible implications for the inter-individual variability. *Foods*, **2020**, *9* (1), 2.

183. de Ferrars, R. M.; Czank, C.; Saha, S.; Needs, P. W.; Zhang, Q.; Raheem, K. S.;

Botting, N. P.; Kroon, P. A.; Kay, C. D. Methods for isolating, identifying, and quantifying anthocyanin metabolites in clinical samples. *Analytical Chemistry*, **2014**, *86* (20), 10052–10058.

184. Ludwig, I. A.; Mena, P.; Calani, L.; Borges, G.; Pereira-Caro, G.; Bresciani, L.; Del Rio, D.; Lean, M. E. J.; Crozier, A. New insights into the bioavailability of red raspberry anthocyanins and ellagitannins. *Free Radical Biology & Medicine*, **2015**, *89*, 758–769.

185. Nordic Nutrition Recommendations 2012 - integrating nutrition and physical activity **2014**, No. 002.

186. Goodman, B. E. Insights into digestion and absorption of major nutrients in humans. *Advances in Physiology Education*, **2010**, *34* (2), 44–53.

187. Wright, E. M.; Martín, M. G.; Turk, E. Intestinal absorption in health and disease—sugars. *Best Practice & Research Clinical Gastroenterology*, **2003**, *17* (6), 943–956.

188. Adisakwattana, S.; Yibchok-Anun, S.; Charoenlertkul, P.; Wongsasiripat, N. Cyanidin-3-rutinoside alleviates postprandial hyperglycemia and its synergism with acarbose by inhibition of intestinal α-glucosidase. *Journal of Clinical Biochemistry and Nutrition*, **2011**, *49* (1), 36–41.

189. Xu, Y.; Xie, L.; Xie, J.; Liu, Y.; Chen, W. Pelargonidin-3-*O*-rutinoside as a novel α-glucosidase inhibitor for improving postprandial hyperglycemia. *Chemical Communications (Cambridge, England)*, **2018**, *55* (1), 39–42.

190. Jayaprakasam, B.; Vareed, S. K.; Olson, L. K.; Nair, M. G. Insulin secretion by bioactive anthocyanins and anthocyanidins present in fruits. *Journal of Agricultural and Food Chemistry*, **2005**, *53* (1), 28–31.

191. Sasaki, R.; Nishimura, N.; Hoshino, H.; Isa, Y.; Kadowaki, M.; Ichi, T.; Tanaka, A.; Nishiumi, S.; Fukuda, I.; Ashida, H.; Horio, F.; Tsuda, T. Cyanidin 3-glucoside ameliorates hyperglycemia and insulin sensitivity due to downregulation of retinol binding protein 4 expression in diabetic mice. *Biochemical Pharmacology*, **2007**, *74* (11), 1619–1627.

192. Guo, H.; Xia, M.; Zou, T.; Ling, W.; Zhong, R.; Zhang, W. Cyanidin-3-glucoside attenuates obesity-associated insulin resistance and hepatic steatosis in high-fat diet-fed and db/db mice via the transcription factor FoxO1. *The Journal of Nutritional Biochemistry*, **2012**, *23* (4), 349–360.

193. Li, D.; Zhang, Y.; Liu, Y.; Sun, R.; Xia, M. Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients. *The Journal of Nutrition*, **2015**, *145* (4), 742–748.

194. Matsui, T.; Ebuchi, S.; Kobayashi, M.; Fukui, K.; Sugita, K.; Terahara, N.; Matsumoto, K. Anti-hyperglycemic effect of diacylated anthocyanin derived from *Ipomoea batatas* cultivar Ayamurasaki can be achieved through the α-glucosidase inhibitory action. *Journal of Agricultural and Food Chemistry*, **2002**, *50* (25), 7244–7248.

195. Jang, H.-H.; Kim, H.-W.; Kim, S.-Y.; Kim, S.-M.; Kim, J.-B.; Lee, Y.-M. *In vitro* and *in vivo* hypoglycemic effects of cyanidin 3-caffeoyl-*p*-hydroxybenzoylsophoroside-5-glucoside, an anthocyanin isolated from purple-fleshed sweet potato. *Food Chemistry*, **2019**, *272*, 688–693.

196. Bell, L.; Lamport, D. J.; Butler, L. T.; Williams, C. M. A study of glycaemic effects following acute anthocyanin-rich blueberry supplementation in healthy young adults. *Food & Function*, **2017**, *8* (9), 3104–3110.

197. Linderborg, K. M.; Järvinen, R.; Lehtonen, H.-M.; Viitanen, M.; Kallio, H. P. T. The fiber and/or polyphenols present in lingonberries null the glycemic effect of the sugars present in the berries when consumed together with added glucose in healthy human volunteers. *Nutrition Research*, **2012**, *32* (7), 471–478.

198. Castro-Acosta, M. L.; Smith, L.; Miller, R. J.; McCarthy, D. I.; Farrimond, J. A.; Hall, W. L. Drinks containing anthocyanin-rich blackcurrant extract decrease postprandial blood glucose, insulin and incretin

concentrations. *The Journal of Nutritional Biochemistry*, **2016**, *38*, 154–161.

199. Törrönen, R.; Kolehmainen, M.; Sarkkinen, E.; Mykkänen, H.; Niskanen, L. Postprandial glucose, insulin, and free fatty acid responses to sucrose consumed with blackcurrants and lingonberries in healthy women. *The American Journal of Clinical Nutrition*, **2012**, *96* (3), 527–533.

200. Törrönen, R.; McDougall, G. J.; Dobson, G.; Stewart, D.; Hellström, J.; Mattila, P.; Pihlava, J.-M.; Koskela, A.; Karjalainen, R. Fortification of blackcurrant juice with crowberry: Impact on polyphenol composition, urinary phenolic metabolites, and postprandial glycemic response in healthy subjects. *Journal of Functional Foods*, **2012**, *4* (4), 746–756.

201. Törrönen, R.; Sarkkinen, E.; Tapola, N.; Hautaniemi, E.; Kilpi, K.; Niskanen, L. Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. *British Journal of Nutrition*, **2010**, *103* (08), 1094–1097.

202. Törrönen, R.; Sarkkinen, E.; Niskanen, T.; Tapola, N.; Kilpi, K.; Niskanen, L. Postprandial glucose, insulin and glucagon-like peptide 1 responses to sucrose ingested with berries in healthy subjects. *British Journal of Nutrition*, **2012**, *107* (10), 1445–1451.

203. Törrönen, R.; Kolehmainen, M.; Sarkkinen, E.; Poutanen, K.; Mykkänen, H.; Niskanen, L. Berries reduce postprandial insulin responses to wheat and rye breads in healthy women. *The Journal of Nutrition*, **2013**, *143* (4), 430–436.

204. Clegg, M. E.; Pratt, M.; Meade, C. M.; Henry, C. J. K. The addition of raspberries and blueberries to a starch-based food does not alter the glycaemic response. *The British Journal of Nutrition*, **2011**, *106* (3), 335–338.

205. Jennings, A.; Welch, A. A.; Spector, T.; Macgregor, A.; Cassidy, A. Intakes of anthocyanins and flavones are associated with biomarkers of insulin resistance and inflammation in women. *The Journal of Nutrition*, **2014**, *144* (2), 202–208.

206. Khoo, H. E.; Azlan, A.; Tang, S. T.; Lim, S. M. Anthocyanidins and anthocyanins: colored pigments as food, pharmaceutical ingredients, and the potential health benefits. *Food & Nutrition Research*, **2017**, *61* (1), 1361779.

207. Castro-Acosta, M. L.; Lenihan-Geels, G. N.; Corpe, C. P.; Hall, W. L. Berries and anthocyanins: promising functional food ingredients with postprandial glycaemia-lowering effects. *The Proceedings of the Nutrition Society*, **2016**, *75* (3), 342–355.

208. Yang, L.; Ling, W.; Du, Z.; Chen, Y.; Li, D.; Deng, S.; Liu, Z.; Yang, L. Effects of anthocyanins on cardiometabolic health: a systematic review and meta-analysis of randomized controlled trials. *Advances in Nutrition (Bethesda, Md.)*, **2017**, *8* (5), 684–693.

209. Rocha, D. M. U. P.; Caldas, A. P. S.; da Silva, B. P.; Hermsdorff, H. H. M.; Alfenas, R. de C. G. Effects of blueberry and cranberry consumption on type 2 diabetes glycemic control: a systematic review. *Critical Reviews in Food Science and Nutrition*, **2019**, *59* (11), 1816–1828.

210. Coe, S.; Ryan, L. Impact of polyphenol-rich sources on acute postprandial glycaemia: a systematic review. *Journal of Nutritional Science*, **2016**, *5*, e24.

211. Ramdath, D. D.; Padhi, E.; Hawke, A.; Sivaramalingam, T.; Tsao, R. The glycemic index of pigmented potatoes is related to their polyphenol content. *Food & Function*, **2014**, *5* (5), 909–915.

212. Ayoub, H. M.; McDonald, M. R.; Sullivan, J. A.; Tsao, R.; Platt, M.; Simpson, J.; Meckling, K. A. The effect of anthocyanin-rich purple vegetable diets on metabolic syndrome in obese Zucker rats. *Journal of Medicinal Food*, **2017**, *20* (12), 1240–1249.

213. Vinson, J. A.; Demkosky, C. A.; Navarre, D. A.; Smyda, M. A. High-antioxidant potatoes: acute *in vivo* antioxidant source and hypotensive agent in humans after supplementation to hypertensive subjects. *Journal of Agricultural and Food Chemistry*, **2012**, *60* (27), 6749–6754.

214. Choi, M.-K.; Park, S.-J.; Eom, S. H.; Kang, M.-H. Anti-diabetic and hypolipidemic

effects of purple-fleshed potato in streptozotocin-induced diabetic rats. *Food Science and Biotechnology*, **2013**, *22* (5), 1–6.

215. Moser, S.; Aragon, I.; Furrer, A.; Van Klinken, J.-W.; Kaczmarczyk, M.; Lee, B.-H.; George, J.; Hamaker, B. R.; Mattes, R.; Ferruzzi, M. G. Potato phenolics impact starch digestion and glucose transport in model systems but translation to phenolic rich potato chips results in only modest modification of glycemic response in humans. *Nutrition Research*, **2018**, *52*, 57–70.

216. Strugała, P.; Dzydzan, O.; Brodyak, I.; Kucharska, A. Z.; Kuropka, P.; Liuta, M.; Kaleta-Kuratewicz, K.; Przewodowska, A.; Michałowska, D.; Gabrielska, J.; Sybirna, N. Antidiabetic and antioxidative potential of the Blue Congo variety of purple potato extract in streptozotocin-induced diabetic rats. *Molecules (Basel, Switzerland)*, **2019**, *24* (17), 3126.

217. Zhao, J.-G.; Yan, Q.-Q.; Lu, L.-Z.; Zhang, Y.-Q. *In vivo* antioxidant, hypoglycemic, and anti-tumor activities of anthocyanin extracts from purple sweet potato. *Nutrition Research and Practice*, **2013**, *7* (5), 359–365.

218. Poudyal, H.; Panchal, S.; Brown, L. Comparison of purple carrot juice and β-carotene in a high-carbohydrate, high-fat diet-fed rat model of the metabolic syndrome. *The British Journal of Nutrition*, **2010**, *104* (9), 1322–1332.

219. Buko, V.; Zavodnik, I.; Kanuka, O.; Belonovskaya, E.; Naruta, E.; Lukivskaya, O.; Kirko, S.; Budryn, G.; Żyżelewicz, D.; Oracz, J.; Sybirna, N. Antidiabetic effects and erythrocyte stabilization by red cabbage extract in streptozotocin-treated rats. *Food & Function*, **2018**, *9* (3), 1850–1863.

220. Estiasih, T.; Umaro, D.; Harijono. Hypoglycemic effect of crude water soluble polysaccharide extracted from tubers of purple and yellow water yam (*Dioscorea alata* L.) on alloxan-induced hyperglycemia Wistar rats. *Progress in Nutrition*, **2018**, *20* (1-S), 59–67.

221. Hansen, K.; Sickelmann, F.; Pietrowsky, R.; Fehm, H. L.; Born, J. Systemic immune changes following meal intake in humans. *The American Journal of Physiology*, **1997**, *273* (2 Pt 2), R548-553.

222. Calder, P. C.; Ahluwalia, N.; Brouns, F.; Buetler, T.; Clement, K.; Cunningham, K.; Esposito, K.; Jönsson, L. S.; Kolb, H.; Lansink, M.; Marcos, A.; Margioris, A.; Matusheski, N.; Nordmann, H.; O'Brien, J.; Pugliese, G.; Rizkalla, S.; Schalkwijk, C.; Tuomilehto, J.; Wärnberg, J.; Watzl, B.; Winklhofer-Roob, B. M. Dietary factors and low-grade inflammation in relation to overweight and obesity. *The British Journal of Nutrition*, **2011**, *106*, S5-78.

223. Calder, P. C.; Albers, R.; Antoine, J.-M.; Blum, S.; Bourdet-Sicard, R.; Ferns, G. A.; Folkerts, G.; Friedmann, P. S.; Frost, G. S.; Guarner, F.; Løvik, M.; Macfarlane, S.; Meyer, P. D.; M'Rabet, L.; Serafini, M.; van Eden, W.; van Loo, J.; Vas Dias, W.; Vidry, S.; Winklhofer-Roob, B. M.; Zhao, J. Inflammatory disease processes and interactions with nutrition. *The British Journal of Nutrition*, **2009**, *101*, S1-45.

224. Muñoz, A.; Costa, M. Nutritionally mediated oxidative stress and inflammation. *Oxidative Medicine and Cellular Longevity*, **2013**, *2013*, 610950.

225. Gregersen, S.; Samocha-Bonet, D.; Heilbronn, L. K.; Campbell, L. V. Inflammatory and oxidative stress responses to high-carbohydrate and high-fat meals in healthy humans. *Journal of Nutrition and Metabolism*, **2012**, *2012*, 238056.

226. Dickinson, S.; Hancock, D. P.; Petocz, P.; Ceriello, A.; Brand-Miller, J. High-glycemic index carbohydrate increases nuclear factor-kappaB activation in mononuclear cells of young, lean healthy subjects. *The American Journal of Clinical Nutrition*, **2008**, *87* (5), 1188–1193.

227. Evans, J. L.; Goldfine, I. D.; Maddux, B. A.; Grodsky, G. M. Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. *Endocrine Reviews*, **2002**, *23* (5), 599–622.

228. Wellen, K. E.; Hotamisligil, G. S. Inflammation, stress, and diabetes. *The*

Journal of Clinical Investigation, **2005**, *115* (5), 1111–1119.

229. Bloch-Damti, A.; Bashan, N. Proposed mechanisms for the induction of insulin resistance by oxidative stress. *Antioxidants & Redox Signaling*, **2005**, *7* (11–12), 1553–1567.

230. Ceriello, A.; Motz, E. Is oxidative stress the pathogenic mechanism underlying insulin resistance, diabetes, and cardiovascular disease? The common soil hypothesis revisited. *Arteriosclerosis, Thrombosis, and Vascular Biology*, **2004**, *24* (5), 816–823.

231. Patel, C.; Ghanim, H.; Ravishankar, S.; Sia, C. L.; Viswanathan, P.; Mohanty, P.; Dandona, P. Prolonged reactive oxygen species generation and nuclear factor-kappaB activation after a high-fat, high-carbohydrate meal in the obese. *The Journal of Clinical Endocrinology and Metabolism*, **2007**, *92* (11), 4476–4479.

232. Dhindsa, S.; Tripathy, D.; Mohanty, P.; Ghanim, H.; Syed, T.; Aljada, A.; Dandona, P. Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-kappaB in mononuclear cells. *Metabolism: Clinical and Experimental*, **2004**, *53* (3), 330–334.

233. Barnes, P. J. Nuclear factor-kappa B. *The International Journal of Biochemistry & Cell Biology*, **1997**, *29* (6), 867–870.

234. Monnier, L.; Mas, E.; Ginet, C.; Michel, F.; Villon, L.; Cristol, J.-P.; Colette, C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA*, **2006**, *295* (14), 1681–1687.

235. Calder, P. C.; Ahluwalia, N.; Albers, R.; Bosco, N.; Bourdet-Sicard, R.; Haller, D.; Holgate, S. T.; Jönsson, L. S.; Latulippe, M. E.; Marcos, A.; Moreines, J.; M'Rini, C.; Müller, M.; Pawelec, G.; van Neerven, R. J. J.; Watzl, B.; Zhao, J. A consideration of biomarkers to be used for evaluation of inflammation in human nutritional studies. *The British Journal of Nutrition*, **2013**, *109*, S1-34.

236. Edirisinghe, I.; Banaszewski, K.; Cappozzo, J.; Sandhya, K.; Ellis, C. L.; Tadapaneni, R.; Kappagoda, C. T.; Burton-Freeman, B. M. Strawberry anthocyanin and its association with postprandial inflammation and insulin. *The British Journal of Nutrition*, **2011**, *106* (6), 913–922.

237. Mathison, B. D.; Kimble, L. L.; Kaspar, K. L.; Khoo, C.; Chew, B. P. Consumption of cranberry beverage improved endogenous antioxidant status and protected against bacteria adhesion in healthy humans: a randomized controlled trial. *Nutrition Research*, **2014**, *34* (5), 420–427.

238. Garcia-Alonso, M.; Minihane, A.-M.; Rimbach, G.; Rivas-Gonzalo, J. C.; de Pascual-Teresa, S. Red wine anthocyanins are rapidly absorbed in humans and affect monocyte chemoattractant protein 1 levels and antioxidant capacity of plasma. *Journal of Nutritional Biochemistry*, **2009**, *20* (7), 521–529.

239. Jin, Y.; Alimbetov, D.; George, T.; Gordon, M. H.; Lovegrove, J. A. A randomised trial to investigate the effects of acute consumption of a blackcurrant juice drink on markers of vascular reactivity and bioavailability of anthocyanins in human subjects. *European Journal of Clinical Nutrition*, **2011**, *65* (7), 849–856.

240. Sardo, C. L.; Kitzmiller, J. P.; Apseloff, G.; Harris, R. B.; Roe, D. J.; Stoner, G. D.; Jacobs, E. T. An open-label randomized crossover trial of lyophilized black raspberries on postprandial inflammation in older overweight males: a pilot study. *American Journal of Therapeutics*, **2016**, *23* (1), e86-91.

241. Ellis, C. L.; Edirisinghe, I.; Kappagoda, T.; Burton-Freeman, B. Attenuation of meal-induced inflammatory and thrombotic responses in overweight men and women after 6-week daily strawberry (Fragaria) intake. A randomized placebo-controlled trial. *Journal of Atherosclerosis and Thrombosis*, **2011**, *18* (4), 318–327.

242. Karlsen, A.; Retterstøl, L.; Laake, P.; Paur, I.; Kjølsrud-Bøhn, S.; Sandvik, L.; Blomhoff, R. Anthocyanins inhibit nuclear factor-κB activation in monocytes and reduce plasma concentrations of pro-inflammatory mediators in healthy adults.

The Journal of Nutrition, **2007**, *137* (8), 1951–1954.

243. Guo, Y.; Zhang, P.; Liu, Y.; Zha, L.; Ling, W.; Guo, H. A dose-response evaluation of purified anthocyanins on inflammatory and oxidative biomarkers and metabolic risk factors in healthy young adults: a randomized controlled trial. *Nutrition*, **2020**, *74*, 110745.

244. Zhu, Y.; Xia, M.; Yang, Y.; Liu, F.; Li, Z.; Hao, Y.; Mi, M.; Jin, T.; Ling, W. Purified anthocyanin supplementation improves endothelial function via NO-cGMP activation in hypercholesterolemic individuals. *Clinical Chemistry*, **2011**, *57* (11), 1524–1533.

245. Zhang, X.; Zhu, Y.; Song, F.; Yao, Y.; Ya, F.; Li, D.; Ling, W.; Yang, Y. Effects of purified anthocyanin supplementation on platelet chemokines in hypocholesterolemic individuals: a randomized controlled trial. *Nutrition & Metabolism*, **2016**, *13*, 86.

246. Joseph, S. V.; Edirisinghe, I.; Burton-Freeman, B. M. Berries: anti-inflammatory effects in humans. *Journal of Agricultural and Food Chemistry*, **2014**, *62* (18), 3886–3903.

247. Fallah, A. A.; Sarmast, E.; Fatehi, P.; Jafari, T. Impact of dietary anthocyanins on systemic and vascular inflammation: systematic review and meta-analysis on randomised clinical trials. *Food and Chemical Toxicology*, **2020**, *135*, 110922.

248. Kaspar, K. L.; Park, J. S.; Brown, C. R.; Mathison, B. D.; Navarre, D. A.; Chew, B. P. Pigmented potato consumption alters oxidative stress and inflammatory damage in men. *The Journal of Nutrition*, **2011**, *141* (1), 108–111.

249. Sproston, N. R.; Ashworth, J. J. Role of C-reactive protein at sites of inflammation and infection. *Frontiers in Immunology*, **2018**, *9*, 754.

250. Deshmane, S. L.; Kremlev, S.; Amini, S.; Sawaya, B. E. Monocyte chemoattractant protein-1 (MCP-1): an overview. *Journal of Interferon & Cytokine Research*, **2009**, *29* (6), 313–326.

251. Marques, R. E.; Guabiraba, R.; Russo, R. C.; Teixeira, M. M. Targeting CCL5 in inflammation. *Expert Opinion on Therapeutic Targets*, **2013**, *17* (12), 1439–1460.

252. Murphy, P. M. CXC Chemokines. In: *Encyclopedia of hormones.* Henry H. L, Norman A. W. (Editors); Academic Press, 2013; pp.351-362.

253. Kucharzik, T.; Hudson, J. T.; Lügering, A.; Abbas, J. A.; Bettini, M.; Lake, J. G.; Evans, M. E.; Ziegler, T. R.; Merlin, D.; Madara, J. L.; Williams, I. R. Acute induction of human IL-8 production by intestinal epithelium triggers neutrophil infiltration without mucosal injury. *Gut*, **2005**, *54* (11), 1565–1572.

254. Janssens, R.; Struyf, S.; Proost, P. The unique structural and functional features of CXCL12. *Cellular & Molecular Immunology*, **2018**, *15* (4), 299–311.

255. Bekisz, J.; Schmeisser, H.; Pontzer, C.; Zoon, K. C. Interferons. In: *Encyclopedia of hormones.* Henry H. L, Norman A. W. (Editors); Academic Press, 2013; pp.397-405.

256. Paul, W. E. Interleukin-4. In: *Encyclopedia of hormones.* Henry H. L, Norman A. W. (Editors); Academic Press, 2013; pp.416-422.

257. Garbers, C.; Heink, S.; Korn, T.; Rose-John, S. Interleukin-6: designing specific therapeutics for a complex cytokine. *Nature Reviews Drug Discovery*, **2018**, *17* (6), 395–412.

258. Couper, K. N.; Blount, D. G.; Riley, E. M. IL-10: The master regulator of immunity to infection. *The Journal of Immunology*, **2008**, *180* (9), 5771–5777.

259. Różańska, D.; Regulska-Ilow, B. The significance of anthocyanins in the prevention and treatment of type 2 diabetes. *Advances in Clinical and Experimental Medicine*, **2018**, *27* (1), 135–142.

260. Sancho, R. A. S.; Pastore, G. M. Evaluation of the effects of anthocyanins in type 2 diabetes. *Food Research International*, **2012**, *46* (1), 378–386.

261. Barik, S. K.; Russell, W. R.; Moar, K. M.; Cruickshank, M.; Scobbie, L.; Duncan, G.; Hoggard, N. The anthocyanins in black currants regulate postprandial hyperglycaemia primarily by inhibiting α-glucosidase while other phenolics modulate salivary α-amylase, glucose uptake and sugar transporters. *The Journal of Nutritional Biochemistry*, **2020**, *78*, 108325.

262. Akkarachiyasit, S.; Charoenlertkul, P.; Yibchok-anun, S.; Adisakwattana, S. Inhibitory activities of cyanidin and its glycosides and synergistic effect with acarbose against intestinal α-glucosidase and pancreatic α-amylase. *International Journal of Molecular Sciences*, **2010**, *11* (9), 3387–3396.

263. Iwai, K.; Kim, M.-Y.; Onodera, A.; Matsue, H. Alpha-glucosidase inhibitory and antihyperglycemic effects of polyphenols in the fruit of *Viburnum dilatatum* Thunb. *Journal of Agricultural and Food Chemistry*, **2006**, *54* (13), 4588–4592.

264. Kaeswurm, J. A. H.; Könighofer, L.; Hogg, M.; Scharinger, A.; Buchweitz, M. Impact of B-ring substitution and acylation with hydroxy cinnamic acids on the inhibition of porcine α-amylase by anthocyanin-3-glycosides. *Foods*, **2020**, *9* (3), 367.

265. Kalita, D.; Holm, D. G.; LaBarbera, D. V.; Petrash, J. M.; Jayanty, S. S. Inhibition of α-glucosidase, α-amylase, and aldose reductase by potato polyphenolic compounds. *PloS One*, **2018**, *13* (1), e0191025.

266. Ho, G. T. T.; Kase, E. T.; Wangensteen, H.; Barsett, H. Phenolic elderberry extracts, anthocyanins, procyanidins, and metabolites influence glucose and fatty acid uptake in human skeletal muscle cells. *Journal of Agricultural and Food Chemistry*, **2017**, *65* (13), 2677–2685.

267. Matsui, T.; Ueda, T.; Oki, T.; Sugita, K.; Terahara, N.; Matsumoto, K. α-Glucosidase inhibitory action of natural acylated anthocyanins. 2. α-Glucosidase inhibition by isolated acylated anthocyanins. *Journal of Agricultural and Food Chemistry*, **2001**, *49* (4), 1952–1956.

268. Hidalgo, J.; Flores, C.; Hidalgo, M. A.; Perez, M.; Yañez, A.; Quiñones, L.; Caceres, D. D.; Burgos, R. A. Delphinol® standardized maqui berry extract reduces postprandial blood glucose increase in individuals with impaired glucose regulation by novel mechanism of sodium glucose cotransporter inhibition. *Panminerva Medica*, **2014**, *56*, 1–7.

269. Hidalgo, J.; Teuber, S.; Morera, F. J.; Ojeda, C.; Flores, C. A.; Hidalgo, M. A.; Núñez, L.; Villalobos, C.; Burgos, R. A. Delphinidin reduces glucose uptake in mice jejunal tissue and human intestinal cells lines through FFA1/GPR40. *International Journal of Molecular Sciences*, **2017**, *18* (4), 750.

270. Itoh, Y.; Kawamata, Y.; Harada, M.; Kobayashi, M.; Fujii, R.; Fukusumi, S.; Ogi, K.; Hosoya, M.; Tanaka, Y.; Uejima, H.; Tanaka, H.; Maruyama, M.; Satoh, R.; Okubo, S.; Kizawa, H.; Komatsu, H.; Matsumura, F.; Noguchi, Y.; Shinohara, T.; Hinuma, S.; Fujisawa, Y.; Fujino, M. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature*, **2003**, *422* (6928), 173–176.

271. Kato, M.; Tani, T.; Terahara, N.; Tsuda, T. The anthocyanin delphinidin 3-rutinoside stimulates glucagon-like peptide-1 secretion in murine GLUTag cell line via the Ca2+/calmodulin-dependent kinase II pathway. *PLoS ONE*, **2015**, *10* (5), e0126157.

272. Rojo, L. E.; Ribnicky, D.; Logendra, S.; Poulev, A.; Rojas-Silva, P.; Kuhn, P.; Dorn, R.; Grace, M. H.; Lila, M. A.; Raskin, I. *In vitro* and *in vivo* anti-diabetic effects of anthocyanins from maqui berry (*Aristotelia chilensis*). *Food Chemistry*, **2012**, *131* (2), 387–396.

273. Uldry, M.; Thorens, B. The SLC2 family of facilitated hexose and polyol transporters. *Pflügers Archiv: European Journal of Physiology*, **2004**, *447* (5), 480–489.

274. Choi, K. H.; Lee, H. A.; Park, M. H.; Han, J.-S. Cyanidin-3-rutinoside increases glucose uptake by activating the PI3K/Akt pathway in 3T3-L1 adipocytes. *Environmental Toxicology and Pharmacology*, **2017**, *54*, 1–6.

275. Scazzocchio, B.; Varì, R.; Filesi, C.; D'Archivio, M.; Santangelo, C.;

Giovannini, C.; Iacovelli, A.; Silecchia, G.; Li Volti, G.; Galvano, F.; Masella, R. Cyanidin-3-*O*-β-glucoside and protocatechuic acid exert insulin-like effects by upregulating PPARγ activity in human omental adipocytes. *Diabetes*, **2011**, *60* (9), 2234–2244.

276. Hardie, D. G.; Hawley, S. A. AMP-activated protein kinase: the energy charge hypothesis revisited. *BioEssays*, **2001**, *23* (12), 1112–1119.

277. Kurth-Kraczek, E. J.; Hirshman, M. F.; Goodyear, L. J.; Winder, W. W. 5' AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes*, **1999**, *48* (8), 1667–1671.

278. Kurimoto, Y.; Shibayama, Y.; Inoue, S.; Soga, M.; Takikawa, M.; Ito, C.; Nanba, F.; Yoshida, T.; Yamashita, Y.; Ashida, H.; Tsuda, T. Black soybean seed coat extract ameliorates hyperglycemia and insulin sensitivity via the activation of AMP-activated protein kinase in diabetic mice. *Journal of Agricultural and Food Chemistry*, **2013**, *61* (23), 5558–5564.

279. Takikawa, M.; Inoue, S.; Horio, F.; Tsuda, T. Dietary anthocyanin-rich bilberry extract ameliorates hyperglycemia and insulin sensitivity via activation of AMP-activated protein kinase in diabetic mice. *The Journal of Nutrition*, **2010**, *140* (3), 527–533.

280. Scazzocchio, B.; Varì, R.; Filesi, C.; Del Gaudio, I.; D'Archivio, M.; Santangelo, C.; Iacovelli, A.; Galvano, F.; Pluchinotta, F. R.; Giovannini, C.; Masella, R. Protocatechuic acid activates key components of insulin signaling pathway mimicking insulin activity. *Molecular Nutrition & Food Research*, **2015**, *59* (8), 1472–1481.

281. Yamauchi, T.; Kamon, J.; Minokoshi, Y.; Ito, Y.; Waki, H.; Uchida, S.; Yamashita, S.; Noda, M.; Kita, S.; Ueki, K.; Eto, K.; Akanuma, Y.; Froguel, P.; Foufelle, F.; Ferre, P.; Carling, D.; Kimura, S.; Nagai, R.; Kahn, B. B.; Kadowaki, T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nature Medicine*, **2002**, *8* (11), 1288–1295.

282. Tsuda, T.; Ueno, Y.; Aoki, H.; Koda, T.; Horio, F.; Takahashi, N.; Kawada, T.; Osawa, T. Anthocyanin enhances adipocytokine secretion and adipocyte-specific gene expression in isolated rat adipocytes. *Biochemical and Biophysical Research Communications*, **2004**, *316* (1), 149–157.

283. Huang, B.; Wang, Z.; Park, J. H.; Ryu, O. H.; Choi, M. K.; Lee, J.-Y.; Kang, Y.-H.; Lim, S. S. Anti-diabetic effect of purple corn extract on C57BL/KsJ db/db mice. *Nutrition Research and Practice*, **2015**, *9* (1), 22–29.

284. Ahmadian, M.; Suh, J. M.; Hah, N.; Liddle, C.; Atkins, A. R.; Downes, M.; Evans, R. M. PPARγ signaling and metabolism: the good, the bad and the future. *Nature Medicine*, **2013**, *19* (5), 557–566.

285. Iwaki, M.; Matsuda, M.; Maeda, N.; Funahashi, T.; Matsuzawa, Y.; Makishima, M.; Shimomura, I. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes*, **2003**, *52* (7), 1655–1663.

286. Yang, Q.; Graham, T. E.; Mody, N.; Preitner, F.; Peroni, O. D.; Zabolotny, J. M.; Kotani, K.; Quadro, L.; Kahn, B. B. Serum retinol binding protein 4 contributes to insulin resistance in obesity and type 2 diabetes. *Nature*, **2005**, *436* (7049), 356–362.

287. Prior, R. L.; Gu, L.; Wu, X.; Jacob, R. A.; Sotoudeh, G.; Kader, A. A.; Cook, R. A. Plasma antioxidant capacity changes following a meal as a measure of the ability of a food to alter in vivo antioxidant status. *Journal of the American College of Nutrition*, **2007**, *26* (2), 170–181.

288. Seymour, E. M.; Warber, S. M.; Kirakosyan, A.; Noon, K. R.; Gillespie, B.; Uhley, V. E.; Wunder, J.; Urcuyo, D. E.; Kaufman, P. B.; Bolling, S. F. Anthocyanin pharmacokinetics and dose-dependent plasma antioxidant pharmacodynamics following whole tart cherry intake in healthy humans. *Journal of Functional Foods*, **2014**, *11*, 509–516.

289. Mertens-Talcott, S. U.; Rios, J.; Jilma-Stohlawetz, P.; Pacheco-Palencia, L. A.; Meibohm, B.; Talcott, S. T.; Derendorf, H.

Pharmacokinetics of anthocyanins and antioxidant effects after the consumption of anthocyanin-rich acai juice and pulp (*Euterpe oleracea* Mart.) in human healthy volunteers. *Journal of Agricultural and Food Chemistry*, **2008**, *56* (17), 7796–7802.

290. Cao, G.; Russell, R. M.; Lischner, N.; Prior, R. L. Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. *The Journal of Nutrition*, **1998**, *128* (12), 2383–2390.

291. Kay, C. D.; Holub, B. J. The effect of wild blueberry (*Vaccinium angustifolium*) consumption on postprandial serum antioxidant status in human subjects. *British Journal of Nutrition*, **2002**, *88* (04), 389–397.

292. Matsumoto, H.; Nakamura, Y.; Hirayama, M.; Yoshiki, Y.; Okubo, K. Antioxidant activity of black currant anthocyanin aglycons and their glycosides measured by chemiluminescence in a neutral pH region and in human plasma. *Journal of Agricultural and Food Chemistry*, **2002**, *50* (18), 5034–5037.

293. Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*, **1996**, *20* (7), 933–956.

294. Wang, H.; Cao, G.; Prior, R. L. Oxygen radical absorbing capacity of anthocyanins. *Journal of Agricultural and Food Chemistry*, **1997**, *45* (2), 304–309.

295. Kähkönen, M. P.; Heinonen, M. Antioxidant activity of anthocyanins and their aglycons. *Journal of Agricultural and Food Chemistry*, **2003**, *51* (3), 628–633.

296. Ali, H. M.; Almagribi, W.; Al-Rashidi, M. N. Antiradical and reductant activities of anthocyanidins and anthocyanins, structure-activity relationship and synthesis. *Food Chemistry*, **2016**, *194*, 1275–1282.

297. Noda, Y.; Kaneyuki, T.; Igarashi, K.; Mori, A.; Packer, L. Antioxidant activity of nasunin, an anthocyanin in eggplant. *Research Communications in Molecular Pathology and Pharmacology*, **1998**, *102* (2), 175–187.

298. Tamura, H.; Yamagami, A. Antioxidative activity of monoacylated anthocyanins isolated from Muscat Bailey A grape. *Journal of Agricultural and Food Chemistry*, **1994**, *42* (8), 1612–1615.

299. Nemś, A.; Pęksa, A.; Kucharska, A. Z.; Sokół-Łętowska, A.; Kita, A.; Drożdż, W.; Hamouz, K. Anthocyanin and antioxidant activity of snacks with coloured potato. *Food Chemistry*, **2015**, *172*, 175–182.

300. Nemś, A.; Pęksa, A. Polyphenols of coloured-flesh potatoes as native antioxidants in stored fried snacks. *LWT - Food Science and Technology*, **2018**, *97*, 597–602.

301. Ombra, M. N.; Fratianni, F.; Granese, T.; Cardinale, F.; Cozzolino, A.; Nazzaro, F. *In vitro* antioxidant, antimicrobial and anti-proliferative activities of purple potato extracts (*Solanum tuberosum* cv Vitelotte noire) following simulated gastro-intestinal digestion. *Natural Product Research*, **2015**, *29* (11), 1087–1091.

302. Kropat, C.; Mueller, D.; Boettler, U.; Zimmermann, K.; Heiss, E. H.; Dirsch, V. M.; Rogoll, D.; Melcher, R.; Richling, E.; Marko, D. Modulation of Nrf2-dependent gene transcription by bilberry anthocyanins *in vivo*. *Molecular Nutrition & Food Research*, **2013**, *57* (3), 545–550.

303. Ma, Q. Role of Nrf2 in oxidative stress and toxicity. *Annual review of pharmacology and toxicology*, **2013**, *53*, 401–426.

304. Han, K.-H.; Sekikawa, M.; Shimada, K.; Hashimoto, M.; Hashimoto, N.; Noda, T.; Tanaka, H.; Fukushima, M. Anthocyanin-rich purple potato flake extract has antioxidant capacity and improves antioxidant potential in rats. *The British Journal of Nutrition*, **2006**, *96* (6), 1125–1133.

305. Ferrari, D.; Speciale, A.; Cristani, M.; Fratantonio, D.; Molonia, M. S.; Ranaldi, G.; Saija, A.; Cimino, F. Cyanidin-3-*O*-glucoside inhibits NF-kB signalling in intestinal epithelial cells exposed to TNF-α and exerts protective effects via Nrf2 pathway activation. *Toxicology Letters*, **2016**, *264*, 51–58.

306. Shih, P.-H.; Yeh, C.-T.; Yen, G.-C. Anthocyanins induce the activation of phase II enzymes through the antioxidant response element pathway against oxidative stress-induced apoptosis. *Journal of Agricultural and Food Chemistry*, **2007**, *55* (23), 9427–9435.

307. Pahl, H. L. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene*, **1999**, *18* (49), 6853–6866.

308. Olejnik, A.; Kowalska, K.; Kidoń, M.; Czapski, J.; Rychlik, J.; Olkowicz, M.; Dembczyński, R. Purple carrot anthocyanins suppress lipopolysaccharide-induced inflammation in the co-culture of intestinal Caco-2 and macrophage RAW264.7 cells. *Food & Function*, **2016**, *7* (1), 557–564.

309. Ju, J.-H.; Yoon, H.-S.; Park, H.-J.; Kim, M.-Y.; Shin, H.-K.; Park, K.-Y.; Yang, J.-O.; Sohn, M.-S.; Do, M.-S. Anti-obesity and antioxidative effects of purple sweet potato extract in 3T3-L1 adipocytes *in vitro*. *Journal of Medicinal Food*, **2011**, *14* (10), 1097–1106.

310. Johnson, G. L.; Lapadat, R. Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinases. *Science*, **2002**, *298* (5600), 1911–1912.

311. Lehtonen, H.-M.; Järvinen, R.; Linderborg, K.; Viitanen, M.; Venojärvi, M.; Alanko, H.; Kallio, H. Postprandial hyperglycemia and insulin response are affected by sea buckthorn (*Hippophaë rhamnoides* ssp. turkestanica) berry and its ethanol-soluble metabolites. *European Journal of Clinical Nutrition*, **2010**, *64* (12), 1465–1471.

312. Faul, F.; Erdfelder, E.; Lang, A.-G.; Buchner, A. G*Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behavior Research Methods*, **2007**, *39* (2), 175–191.

313. Faul, F.; Erdfelder, E.; Buchner, A.; Lang, A.-G. Statistical power analyses using G*Power 3.1: tests for correlation and regression analyses. *Behavior Research Methods*, **2009**, *41* (4), 1149–1160.

314. Heinonen, J.; Farahmandazad, H.; Vuorinen, A.; Kallio, H.; Yang, B.; Sainio, T. Extraction and purification of anthocyanins from purple-fleshed potato. *Food and Bioproducts Processing*, **2016**, *99*, 136–146.

315. Tiitinen, K. M.; Yang, B.; Haraldsson, G. G.; Jonsdottir, S.; Kallio, H. P. Fast analysis of sugars, fruit acids, and vitamin C in sea buckthorn (*Hippophaë rhamnoides* L.) varieties. *Journal of Agricultural and Food Chemistry*, **2006**, *54* (7), 2508–2513.

316. Zheng, J.; Kallio, H.; Linderborg, K.; Yang, B. Sugars, sugar alcohols, fruit acids, and ascorbic acid in wild Chinese sea buckthorn (*Hippophaë rhamnoides* ssp. sinensis) with special reference to influence of latitude and altitude. *Food Research International*, **2011**, *44* (7), 2018–2026.

317. Sandell, M.; Laaksonen, O.; Järvinen, R.; Rostiala, N.; Pohjanheimo, T.; Tiitinen, K.; Kallio, H. Orosensory profiles and chemical composition of black currant (*Ribes nigrum*) juice and fractions of press residue. *Journal of Agricultural and Food Chemistry*, **2009**, *57* (9), 3718–3728.

318. Lätti, A. K.; Riihinen, K. R.; Kainulainen, P. S. Analysis of anthocyanin variation in wild populations of bilberry (*Vaccinium myrtillus* L.) in Finland. *Journal of Agricultural and Food Chemistry*, **2008**, *56* (1), 190–196.

319. Määttä, K.; Kamal-Eldin, A.; Törrönen, R. Phenolic compounds in berries of black, red, green, and white currants (*Ribes* sp.). *Antioxidants & Redox Signaling*, **2001**, *3* (6), 981–993.

320. Liu, P.; Kallio, H.; Yang, B. Flavonol glycosides and other phenolic compounds in buds and leaves of different varieties of black currant (*Ribes nigrum* L.) and changes during growing season. *Food Chemistry*, **2014**, *160*, 180–189.

321. Cevallos-Casals, B. A.; Cisneros-Zevallos, L. Stoichiometric and kinetic studies of phenolic antioxidants from Andean purple corn and red-fleshed sweetpotato. *Journal of Agricultural and Food Chemistry*, **2003**, *51* (11), 3313–3319.

322. R Core Team. *R: A Language and Environment for Statistical Computing*; 2018.

323. Torchiano, M. *Effsize: Efficient Effect Size Computation. R Package 0.7.4.; 2018.*

324. Warnes, G. R.; Bolker, B.; Bonebakker, L.; Gentleman, R.; Huber, W.; Liaw, A.; Lumley, T.; Maechler, M.; Magnusson, A.; Moeller, S.; Schwartz, M.; Venables, B. *Gplots: Various R Programming Tools for Plotting Data. R Package Version 3.0.1.1.*; 2019.

325. Neuwirth, E. *RColorBrewer: ColorBrewer Palettes. R Package Version 1.1.2.*; 2014.

326. Jokioja, J.; Linderborg, K. M.; Kortesniemi, M.; Nuora, A.; Heinonen, J.; Sainio, T.; Viitanen, M.; Kallio, H.; Yang, B. Anthocyanin-rich extract from purple potatoes decreases postprandial glycemic response and affects inflammation markers in healthy men. *Food Chemistry*, **2020**, 125797.

327. Linderborg, K. M.; Salo, J. E.; Kalpio, M.; Vuorinen, A. L.; Kortesniemi, M.; Griinari, M.; Viitanen, M.; Yang, B.; Kallio, H. Comparison of the postprandial effects of purple-fleshed and yellow-fleshed potatoes in healthy males with chemical characterization of the potato meals. *International Journal of Food Sciences and Nutrition*, **2016**, *67* (5), 581–591.

328. Steinberg, H. O.; Stentz, F. B.; Shankar, N. K. Effects of a high-carb vs. high-fat meal on glycemia, insulin, interleukin-6, TNF-alpha, and apo-B. *Diabetes*, **2018**, *67*, 265-LB.

329. Morton, G. J.; Matsen, M. E.; Bracy, D. P.; Meek, T. H.; Nguyen, H. T.; Stefanovski, D.; Bergman, R. N.; Wasserman, D. H.; Schwartz, M. W. FGF19 action in the brain induces insulin-independent glucose lowering. *The Journal of Clinical Investigation*, **2013**, *123* (11), 4799–4808.

330. Fu, L.; John, L. M.; Adams, S. H.; Yu, X. X.; Tomlinson, E.; Renz, M.; Williams, P. M.; Soriano, R.; Corpuz, R.; Moffat, B.; Vandlen, R.; Simmons, L.; Foster, J.; Stephan, J.-P.; Tsai, S. P.; Stewart, T. A. Fibroblast growth factor 19 increases metabolic rate and reverses dietary and leptin-deficient diabetes. *Endocrinology*, **2004**, *145* (6), 2594–2603.

331. Potthoff, M. J.; Boney-Montoya, J.; Choi, M.; He, T.; Sunny, N. E.; Satapati, S.; Suino-Powell, K.; Xu, H. E.; Gerard, R. D.; Finck, B. N.; Burgess, S. C.; Mangelsdorf, D. J.; Kliewer, S. A. FGF15/19 regulates hepatic glucose metabolism by inhibiting the CREB-PGC-1α pathway. *Cell Metabolism*, **2011**, *13* (6), 729–738.

332. Kir, S.; Kliewer, S. A.; Mangelsdorf, D. J. Roles of FGF19 in liver metabolism. *Cold Spring Harbor Symposia on Quantitative Biology*, **2011**, *76*, 139–144.

333. Aqeel, M.; Forster, A.; Richards, E. A.; Hennessy, E.; McGowan, B.; Bhadra, A.; Guo, J.; Gelfand, S.; Delp, E.; Eicher-Miller, H. A. The effect of timing of exercise and eating on postprandial response in adults: a systematic review. *Nutrients*, **2020**, *12* (1).

334. Kennedy, J. W.; Hirshman, M. F.; Gervino, E. V.; Ocel, J. V.; Forse, R. A.; Hoenig, S. J.; Aronson, D.; Goodyear, L. J.; Horton, E. S. Acute exercise induces GLUT4 translocation in skeletal muscle of normal human subjects and subjects with type 2 diabetes. *Diabetes*, **1999**, *48* (5), 1192–1197.

335. Diamond, M. P.; Simonson, D. C.; DeFronzo, R. A. Menstrual cyclicity has a profound effect on glucose homeostasis. *Fertility and Sterility*, **1989**, *52* (2), 204–208.

336. Östman, E.; Granfeldt, Y.; Persson, L.; Björck, I. Vinegar supplementation lowers glucose and insulin responses and increases satiety after a bread meal in healthy subjects. *European Journal of Clinical Nutrition*, **2005**, *59* (9), 983–988.

337. Santos, H. O.; de Moraes, W. M. A. M.; da Silva, G. A. R.; Prestes, J.; Schoenfeld, B. J. Vinegar (acetic acid) intake on glucose metabolism: A narrative review. *Clinical Nutrition ESPEN*, **2019**, *32*, 1–7.

338. Bassoli, B. K.; Cassolla, P.; Borba-Murad, G. R.; Constantin, J.; Salgueiro-Pagadigorria, C. L.; Bazotte, R. B.; da Silva, R. S. dos S. F.; de Souza, H. M. Chlorogenic acid reduces the plasma glucose peak in the oral glucose tolerance test: effects on hepatic glucose release and glycaemia. *Cell Biochemistry and Function*, **2008**, *26* (3), 320–328.

APPENDIX: ORIGINAL PUBLICATIONS

I Reprinted from *International Journal of Food Sciences and Nutrition* 2016, 67 (5), 581–591, with permission from Taylor & Francis.

II Reprinted from *Food Chemistry* 2020, 310, 125797, 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


UNIVERSITY
OF TURKU