NMR Metabolomics of Foods – Investigating the Influence of Origin on Sea Buckthorn Berries, Brassica Oilseeds and Honey

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Department of Biochemistry
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The origin of foods plays an important role in their metabolome (the set of compounds present as products of metabolic events). The compositions of food plants are inevitably determined by a number of inherent and external factors – most importantly by the genotype (species, subspecies, cultivar, variety) and the prevailing conditions and weather parameters at each growth environment. The declaration of food origin can be defined and protected by law. The constantly increasing consumer awareness towards food origin, authenticity and quality has set the need for efficient tools for their verification. Metabolomics based on nuclear magnetic resonance (NMR) spectroscopy is increasingly being applied in analysing food composition and quality and in detecting food frauds and adulterations.

The aim of the current work was to determine the influence of origin-related variables in food composition and quality by using $^1$H NMR metabolomics. The model foods – sea buckthorn (Hippophaë rhamnoides) berries, oilseeds of Brassica spp. and varietal honey – represent different foods with special sensory, nutritional, bioactive, commercial and national significance. The sea buckthorn berry metabolites were investigated in respect to the genotype (subspecies, cultivar) and geographical origin, with special emphasis on the influence of northern latitudes and related conditions. In the oilseeds, the interspecies variation and the influence of environmental and developmental stage on the seed composition was investigated. NMR profiling was applied in characterising the marker compounds for different honey types for botanical authentication. Multivariate analysis methods such as principal component (PCA) and discriminant analyses (PLS-DA, OPLS-DA) were applied in every sub-study to determine the key metabolites and origin-related factors characterising the food samples.

The sea buckthorn subspecies were mainly distinguished by the relatively high content of ethyl-$\beta$-D-glucopyranoside (ssp. rhamnoides) and malic acid and vitamin C (ssp. sinensis). The northern latitude and respective conditions (the length of growth season, temperature, radiation and precipitation) was shown to alter the chemical composition of berries of the same genetic origin. In subarctic latitudes, the berries formed more ascorbic acid while the levels of ethyl glucose remained relatively low. The berries of cultivar 'Tytti' contained more ethyl glucoside while the berries of 'Terhi' contained more quinic acid in comparison. Calculated from the start of the growing season until harvest, the effective temperature sum (degree days) and the radiation sum correlated positively with ethyl glucoside that accumulated up to six-fold in overripe berries in southern Finland. The sea buckthorn berries (ssp. sinensis) grown at over 2000 m altitude contained typically more ascorbic and malic acids.
The seeds of turnip rape was characterised by a relatively higher sucrose and polyunsaturated fatty acid content over oilseed rape that had a higher content of sinapine and oil in general. Growth conditions with reduced temperature added to the level of unsaturation in the oilseed lipids and delayed the seed development.

The varietal honeys were classified with the aid of NMR profiling, as the typical sugar composition and other botanical markers were characterised. Also, previously unreported markers were designated for dandelion honeys.

The correlations between complex food metabolomes and the origin-related variables were easily accomplished with NMR metabolomics. Especially, the effect of northern conditions on the growth place-dependent compositional flexibility (phenotypic plasticity) of the plant foods was deemed considerable. The results of this thesis can be further used to determine food quality, origin and authenticity and as an aid in plant breeding operations.
SUOMENKIELINEN ABSTRAKTI


Varsinkin ydinmagneettista resonanssispektroskopiaan (NMR) perustuvaa metabolomiikkatutkimusta hyödynnetään yhä useammin elintarvikkeiden koostumukseen, laadun ja aitouden analysoinnissa. Tämän tutkimuksen tarkoituksena oli selvittää alkuperän vaikutusta tyrnimarjojen (Hippophaë rhamnoides), rypsin- ja rapsinsiementen (Brassica spp.) sekä lajihunajan koostumukseen 1H-NMR-metabolomiikan avulla. Nämä elintarvikkeet ovat kansallisesti ja kaupallisesti arvokkaita ja mielenkiintoisia niille tyyppillisten aisteittavien, ravitsemuksellisten ja bioaktiivisten ominaisuuksien ansiosta.


Tyrnin alalajit erottuivat pääasiassa suhteellisesti korkean etyyli-β-D-glukopyranosidin (ssp. rhamnoides) sekä omenahappo- ja C-vitamiinipitoisuuden (ssp. sinensis) perusteella. Pohjoisen leveysasteen ja sille tyyppillisten olosuhteiden (kasvukauden pituus, lämpötila, säteily, sademäärä) todettiin muokanneen samaa geneettistä alkuperää olevien marjojen kemiallista koostumusta. Subarktisilla leveyskaalla tyrnimarjaan muodostui enemmän askorbiinihappoa ja etyylilukuksidin määrä oli alhainen. 'Tytti'-lajikkeen marjat sisälsivät enemmän etyylilukuksidia, kun taas 'Terhi' sisälsi vastavasti enemmän kviinihappoa. Kasvukauden tehoisa lämpösumma ja säteilysumma korreloivat positiivisesti etyylilukuksidin kanssa, jota kertyi ylikysiin

Suhteellisesti korkeampi sakkaroosipitoisuus ja monityydyttymättömien rasvahappojen osuus oli tyypilisempi rypsille, kun taas rapsi erottui rypsinä korkeamman öljy- ja sinapiinipitoisuuden perusteella. Kylmempänä kasvupaikka lisäsi monityydyttymättömien rasvahappojen osuuttaöljysiemennissä ja hidasti siemenen kehittymistä.

NMR-profiloinnin avulla lajihunajat pystyttiin luokittelemaan kullekin hunajalle ominaisen sokerikoostumukseen ja muiden kasvialkooperaatio kertovien merkkiyhdisteiden perusteella. Voikukkahunajalle tunnistettiin myös aiemmin raportoimattomia merkkiyhdisteitä.

NMR-metabolomiikan avulla pystyttiin helposti selvittämään monimutkaisten aineenvaihduntatuotteiden kokonaisuuksien ja elintarvikkeen alkuperään liittyvien muuttujien välisiä yhteyksiä. Varsinkin pohjoisten kasvuolosuhteiden vaikutus kasviperäisten elintarvikkeiden koostumukselliseen vaihtelun oli huomattava. Väitöskirjan tuloksia voidaan hyödyntää elintarvikkeiden laadun, alkuperän ja aitouden varmistamisessa sekä kasvinjalostuksen apuna.
### LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^1$H</td>
<td>Proton</td>
</tr>
<tr>
<td>$^{13}$C</td>
<td>Carbon-13</td>
</tr>
<tr>
<td>Ala</td>
<td>Alanine</td>
</tr>
<tr>
<td>ALA</td>
<td>$\alpha$-Linolenic acid</td>
</tr>
<tr>
<td>CDCl$_3$</td>
<td>Deuterated chloroform (chloroform-$d$)</td>
</tr>
<tr>
<td>CD$_3$OD</td>
<td>Deuterated methanol (methanol-$d_4$)</td>
</tr>
<tr>
<td>COSY</td>
<td>Correlation spectroscopy</td>
</tr>
<tr>
<td>cv.</td>
<td>Cultivar</td>
</tr>
<tr>
<td>d1</td>
<td>Relaxation delay (s)</td>
</tr>
<tr>
<td>D$_2$O</td>
<td>Deuterated water</td>
</tr>
<tr>
<td>DMSO-$d_6$</td>
<td>Deuterated dimethyl sulfoxide (dimethyl sulfoxide-$d_6$)</td>
</tr>
<tr>
<td>ds</td>
<td>Number of dummy scans</td>
</tr>
<tr>
<td>DSS</td>
<td>4,4-dimethyl-4-silapentane-1-sulfonic acid</td>
</tr>
<tr>
<td>FID</td>
<td>Free induction decay</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>HMBC</td>
<td>Heteronuclear multiple-bond correlation</td>
</tr>
<tr>
<td>HMF</td>
<td>Hydroxymethylfurfural</td>
</tr>
<tr>
<td>HR-MAS</td>
<td>High-resolution magic angle spinning</td>
</tr>
<tr>
<td>HSQC</td>
<td>Heteronuclear single-quantum coherence</td>
</tr>
<tr>
<td>Hz</td>
<td>Hertz</td>
</tr>
<tr>
<td>MHz</td>
<td>Megahertz</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>NOESY</td>
<td>Nuclear Overhauser effect spectroscopy</td>
</tr>
<tr>
<td>ns</td>
<td>Number of scans</td>
</tr>
<tr>
<td>OPLS-DA</td>
<td>Orthogonal partial least squares discriminant analysis</td>
</tr>
<tr>
<td>$p$</td>
<td>Loadings matrix</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PLS-DA</td>
<td>Partial least squares (projection to latent structures) discriminant analysis</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>PUFA</td>
<td>Polyunsaturated fatty acid</td>
</tr>
<tr>
<td>$Q^2$</td>
<td>An estimate of predictive ability</td>
</tr>
<tr>
<td>$Q^2_{(cum)}$</td>
<td>Cumulative $Q^2$</td>
</tr>
<tr>
<td>$R^2$</td>
<td>An estimate of goodness of fit</td>
</tr>
<tr>
<td>$R^2_X$</td>
<td>Fraction of $X$ variation explained by a component</td>
</tr>
<tr>
<td>$R^2_{X_{(cum)}}$</td>
<td>Cumulative $R^2_X$</td>
</tr>
<tr>
<td>$R^2_Y$</td>
<td>Fraction of $Y$ variation explained by a component</td>
</tr>
<tr>
<td>$R^2_{Y_{(cum)}}$</td>
<td>Cumulative $R^2_Y$</td>
</tr>
<tr>
<td>RF</td>
<td>Radio-frequency</td>
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### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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</thead>
<tbody>
<tr>
<td>sn</td>
<td>Stereospecific numbering</td>
</tr>
<tr>
<td>S/N</td>
<td>Signal-to-noise ratio</td>
</tr>
<tr>
<td>t</td>
<td>Score matrix</td>
</tr>
<tr>
<td>T</td>
<td>Temperature (K/° C)</td>
</tr>
<tr>
<td>TAG</td>
<td>Triacylglycerol</td>
</tr>
<tr>
<td>TMS</td>
<td>Tetramethylsilane</td>
</tr>
<tr>
<td>TOCSY</td>
<td>Total correlation spectroscopy</td>
</tr>
<tr>
<td>TSP</td>
<td>3-(Trimethylsilyl)propionic-2,2,3,3-$d_4$ acid</td>
</tr>
<tr>
<td>UV</td>
<td>Ultra-violet / unit variance</td>
</tr>
<tr>
<td>VIP</td>
<td>Variable influence on projection</td>
</tr>
<tr>
<td>WAF</td>
<td>Weeks after flowering</td>
</tr>
</tbody>
</table>
LIST OF ORIGINAL PUBLICATIONS


1 INTRODUCTION

Food, as it ultimately consists of tissues, cells, organs or organisms of animal, plant and/or microbial origin, and of the products of their primary and secondary metabolism, is susceptible to numerous endo- and exogenous factors influencing its composition and quality. The origin of a food or a raw ingredient is one of the key elements that influence the occurrence and concentration of food metabolites. From the genetic foundation\textsuperscript{1-5}, growth place\textsuperscript{6-10}, weather and environmental conditions\textsuperscript{3,11-15}, soil\textsuperscript{16,17}, developmental stage\textsuperscript{18-21}, agricultural practices\textsuperscript{22,23} and harvest\textsuperscript{24-28} to processing and storage\textsuperscript{29,30}, the colour, structure, taste, flavour, shelf life and the nutritional quality of the food can be affected in every step of its life cycle (Figure 1). Here, the effect of origin, covering genotype, geographical origin, related abiotic stress and time-related variation is reviewed.

As this thesis and literature review mainly focuses on plants, plant-based foods and agri-foods, the food genotype – referring to the species, subspecies, variety, cultivar and breed – can be regarded to be one of the most influential origin-related factors affecting food composition. The genetic background usually reflects the quantitative, qualitative and ecological values of the food or food crop. Often, the effect of both the genotype and the environment (the genotype × environment interactions) are taken into consideration when explaining phenological traits of (food) plants.\textsuperscript{2,9,17,31} The growth environment dictates the conditions, under which the crops must develop, grow, reproduce and make harvest. The fluctuating weather conditions and changing climate cause seasonal variation in botanic foodstuffs. Environmental stress factors can have an adverse effect on crop productivity and quality. Alternatively, stress may promote the accumulation of plant protectants, also beneficial to human health.\textsuperscript{7,10,32}

As Finland is situated roughly between 60 and 70 degrees northern latitude, its location-related characteristics in e.g. angle of solar radiation, combined with the temperate effects of the Gulf Stream and the prevailing boreal/arctic biotope, create exceptional prerequisites for studying the “northern effect” within one country. Plant-wise, the growing locations north of the Arctic Circle (66° N) are subject to extreme daily light conditions during the summer months. This characteristic is linked to a higher bioactive and nutritional value of food plants as they protect themselves by synthesising secondary metabolites.\textsuperscript{7,32-34}

The model foods chosen for the study – sea buckthorn (\textit{Hippophaë rhamnoides}) berries, oilseeds \textit{Brassica} and honey – represent different Nordic foods and raw materials with special nutritional, sensory, bioactive and commercial value. Sea buckthorn berries, although extensively studied during
**Fig. 1** Factors affecting the composition and quality of (plant) foods. The effect can be seen in crop yield, morphology, sensory properties and nutritional and bioactive value.
the past decades, are still of scientific interest as a high-value berry for direct consumption and the processing of value-added products (foods, nutraceuticals and cosmetics). The oilseed rape (*Brassica napus*) and turnip rape (*Brassica rapa*) allow the self-reliant production of omega-3-rich vegetable oils and sustainable plant protein in Finland. As northern conditions are more favourable to turnip rape, it is more widely cultivated in Finland compared to oilseed rape. The national oilseed production can, however, hang in the balance for changing climate and banning of commonly used yet controversial insecticides. Honey again, is a complex natural product crafted by the honeybees (*Apis mellifera* L.). The composition of honey reflects the botanical and geographical origin of the raw material, the floral nectar, or the honeydew, the sugary secretions of sap-sucking insects. Finnish honey is the northernmost honey in the world. Of the approximate production of 1.6 million kilograms of honey in Finland annually, a fraction is labelled as varietal (unifloral) honey having the sensory, physicochemical and melissopalynological characteristics of a certain plant source. The raw material originating from the nectarous plants or from the nectar-sucking insects producing honeydew, but also the bee metabolism and the conditions during honey processing and storage affect the honey composition.

Metabolomics offers the potential for a universal and holistic approach in food analysis in order to advance food authenticity, traceability, quality and safety and to understand the biological mechanisms and derived from the origin-related prerequisites.\textsuperscript{35-40} Nuclear magnetic resonance (NMR) spectroscopy combined with multivariate data analysis allows the examination of a wide spectrum of compounds at once while extracting the essence of the origin-related information.

The thesis reviews the current literature related to the effects of genotype, geographical origin, abiotic stress and temporal variation on food composition and quality, with special emphasis on sea buckthorn berries, *Brassica* oilseeds and honey. The basics and the recent applications of NMR spectroscopy and metabolomics in food analysis are also covered.
2 REVIEW OF THE LITERATURE

2.1 The effect of origin and related factors on food composition and quality

2.1.1 Genotype and phenotype

The crop genotype can generally be associated with phenological traits such as morphological and sensory properties, crop yield and yield stability. The complex interaction of components from all the functional levels (genome, transcriptome, proteome and metabolome) and the environment produces the phenotype, the output of the system measured in systems-level metabolomics and systems biology. Novel phenotypic traits can be developed through breeding and genetic modification.

According to the NCBI database, the genome sequencing has been carried out for several food plants, including apple (*Malus domestica*), camelina (*Camelina sativa*), chickpea (*Cicer arietinum*), corn (*Zea mays*), cucumber (*Cucumis sativus*), beet (*Beta vulgaris ssp. vulgaris*), field mustard (*Brassica rapa*), mung bean (*Vigna radiata*), muskmelon (*Cucumis melo*), potato (*Solanum tuberosum*), rape (*Brassica napus*), soybean (*Glycine max*), sweet orange (*Citrus sinensis*), tomato (*Solanum lycopersicum*) and wine grape (*Vitis vinifera*). Oilseed rape is one of the food crops that have gone through extensive breeding to optimise yield and nutritional quality. The target traits have been the levels of oil, protein and oleic acid and erucic acid and glucosinolates, the
anti-nutrients. The bred cultivars are now mostly erucic acid-free, low-glucosinolate and high-oleic cultivars. The oil content for example, is a complex quantitative trait, which is linked with other storage and structural compounds in the seed, and is influenced by seed development and environmental conditions.\textsuperscript{48} The walnut (\textit{Juglans regia} L.) genotype has been shown to affect mainly the fatty acids, hydrocarbons and sterols in the nut oil. The established fingerprints for oil of three walnut varieties were cycloartenol, eicosane and tetracosane (var. 'Criolla'), linolenic acid, 24-methylenecycloartanol and tetradecane ('Chandler'), and docosane and oleic acid ('Franquette').\textsuperscript{49}

In blackcurrants (\textit{Ribes nigrum} L.), the genotype was shown to be the most significant factor for the berry composition.\textsuperscript{4} The highest variation in anthocyanins, ascorbic acid (vitamin C), flavonols, phenolic acids, sugars and titratable acidity was seen among different selections and cultivars. Of Finnish blackcurrant cultivars, 'Melalahti' has a high content of glucose and a ratio of sugars and acids compared to varieties 'Mortti' and 'Ola', which had relatively higher levels of fructose, citric acid, quinic acid and ascorbic acid.\textsuperscript{33} Similarly, the strawberry (\textit{Fragaria} spp.) genotype affects both the sensory and bioactive properties of the fruit, for example, the volatile compounds contributing to the aroma.\textsuperscript{1,23,50} Capocasa et al. reported that the strawberry genotype is more important to the nutritional quality of the fruit than the cultivation conditions.\textsuperscript{1} Peach (\textit{Prunus persicum}) cultivar with resistance to biotic stress (herbivory) shows higher levels of volatile compounds compared to a vulnerable cultivar.\textsuperscript{51} In apples, the 'Almagold' cultivar, resistant to apple scab (\textit{Venturia inaequalis}), exhibit comparatively higher antifungal properties than the more susceptible variety, 'Golden Delicious'.\textsuperscript{52}

Food genotype can sometimes be a matter of authenticity and consumer safety. Due to recent food scandals, for example the European horse meat scam in 2013, the awareness towards food origin and authenticity and the need for efficient tools to verify them have increased. Also, the public is concerned about the potential threats of genetically modified (GM) crops to food safety and food security. Food metabolomics, DNA-based methods and other tools have been developed to identify and detect fraudulent and genetically modified foods from authentic and non-GM products, respectively. Multiplex polymerase chain reaction (PCR) technique, detecting multiple target sequences simultaneously, has been used to detect and identify genetically modified crops such as soybean, maize and canola.\textsuperscript{53}

The authenticity of meat, fish and the products thereof is usually determined at species level. For example, different NMR-based methods have been developed to authenticate beef from horse meat by their triacylglycerol profiles.\textsuperscript{54} The origin of fish (wild vs. cultured) has been studied on e.g. sea
bass (*Dicentrarchus labrax*)\textsuperscript{55} and gilthead sea bream (*Sparus aurata*)\textsuperscript{56}.

### 2.1.2 Geographical origin

The geographical origin refers to a specific country, region, growth site or latitude. The altitude and topography are also included in this retrospect. The term *terroir*, often used in oenology and viticulture, describes the effect of origin and related factors on the metabolic characteristics of wine grapes (*Vitis vinifera* L.) and wine.\textsuperscript{17} Largely, *terroir* refers to a specific region and the surrounding ecosystem and related environmental conditions. The effect of cultivar, viticultural practices, edaphic factors and the topography of the vineyard can be sometimes be covered by this term.\textsuperscript{17} Certain origin-specific food names are protected in the EU.

The growth conditions can vary substantially at different latitudes. Especially, latitude affects the length of growth season, day length, the intensity and quality of light, and temperature.\textsuperscript{7} At high latitudes, the growth conditions may cause plants abiotic stress, affecting their development and metabolism. Same genotypes can produce different phenotypes in different conditions, exhibiting plasticity. In the Nordic countries, where the vegetative period is generally short, the growing season is defined as the period when the daily mean temperature is above +5 °C.\textsuperscript{57} The long summer days and cool night temperatures characteristic to northern climate have been shown to promote biosynthesis of phenolic compounds in plants.\textsuperscript{7} Cool climate and winter can also reduce the prevalence of pests and subsequently the need for their chemical control. The differences in longitude, again, may represent varying topography, elevation/altitude, microclimates, biotope, or agricultural zone. Out of these, the altitude can again shift the environmental conditions to a more unfavourable direction as it increases.

Element- and natural abundance isotope-based analyses are prominent in tracing the geographical origin of foods.\textsuperscript{58} Of mass spectrometric techniques, the isotope ratio (IR-MS), inductively coupled plasma (ICP-MS), proton transfer reaction (PTR-MS) and gas chromatography–mass spectrometry (GC-MS) have been applied in geographical authentication due to their high sensitivity when analysing *e.g.* inorganic elements and volatiles.\textsuperscript{59} On the other hand, robust NMR-based analyses combined with chemometrics efficiently discriminate different foods based on their geographical origin, covering a wide range of metabolites in one analysis.\textsuperscript{8,59-64} As an example, the natural distribution of C\textsubscript{3} and C\textsubscript{4} plants at higher and lower latitudes, respectively, affect the $^{13}$C/$^{12}$C gradient in the plant material which can be used as an indicator of geographical origin.\textsuperscript{58}

As the response of fifteen basil (*Ocimum* spp.) genotypes (representing five
species) to their genotype–environment interactions was studied, the yield of fresh herb and essential oil was shown to be strongly influenced by the environmental conditions at different locations.\textsuperscript{2} The two agroclimates compared produced seven different chemotypes, all of which were characterised by specific volatile fingerprints. In 1977, Härđh & Härđh showed that northern latitude in Finland lowered the content of carotene in carrot (\textit{Daucus carota} L.) and parsley (\textit{Petroselinum crispum}), and intensified the colours in strawberry, tomato, beet root, spinach (\textit{Spinacia oleracea}) and lettuce (\textit{Lactuca sativa}).\textsuperscript{65} Also, carrot, beet root, swede (\textit{Brassica napus} subsp. \textit{rapifera}) and strawberries exhibited higher content of sugar and dry matter in the north (67–69° N) compared to south (60° N). The comparison of blackcurrants grown in southern (56° N) and northern (65° N) Sweden showed that the levels of phenolic acids and glucosides of cyanidin, quercetin and kaempferol were higher in the north.\textsuperscript{4} With Finnish blackcurrants, lower amounts of total flavonol glycosides, anthocyanins and phenolics was recorded in the northern berries (regardless of cultivar) contrary to the higher amount of hydroxycinnamic acid conjugates (cv. 'Mortti' and 'Ola').\textsuperscript{12} High latitude also correlated with lower content of sugars and citric acid and higher content of malic, quinic and ascorbic acids in the blackcurrant juice.\textsuperscript{33}

Statistically significant differences can be seen in the content of palmitoleic acid, sterols, triterpenic alcohols and hydrocarbons in virgin olive oils (VOOs) from different altitudinal origins.\textsuperscript{66} Higher level of $\beta$-sitosterol is characteristic to VOOs from low-altitude (< 400 m) and 24-methylenecycloarhthanol to VOOs from high altitude (> 700 m). In sea buckthorn (\textit{Hippophaë rhamnoides} ssp. \textit{sinensis}) berries, high altitude has been shown to decrease the content of sugars while increasing malic and ascorbic acids.\textsuperscript{67}

Cocoa (\textit{Theobroma cacao} L.) beans can be traced to originate from Africa, America or Asia and Oceania based on their compositional characteristics. However, the differences are also linked to the local varieties or hybrids and the techniques used in cocoa processing and fermentation. According to Marseglia et al., the American cocoa beans can be characterised by caffeine, caffeic acid, acetic acid, epicatechin and amino acids, compared to African beans that have higher levels of citric acid, formic acid and sugars.\textsuperscript{64} The elucidation of the Asian and Oceanian samples was not, however, as clear-cut, possibly due to their close genetic background to the cocoa beans from Africa. Chocolate made from the cocoa beans from Ghana and Nigeria has been shown to correlate with strongly perceived chocolate flavour, showing the influence of the country of origin on the flavour characteristics and quality of chocolate.\textsuperscript{6} The knowledge of the quality properties of foods from certain regions may steer consumer choices.

In honey, the geographical origin is closely related to the botanical origin
due the regional differences in local flora.\textsuperscript{61} The physicochemical and sensory properties of honey are highly dependent on the origin of the raw material, the floral nectar or the honeydew produced by nectar-feeding insects.\textsuperscript{68} For example, the colour, consistency, aroma and sugar composition of the honey are mostly determined by the nectar source. The geographical origin \textit{per se} may have an effect on the moisture content, acidity and the level of active enzymes in honey. Also, the stable isotope abundance ratios or the mineral and trace element compositions can be specific to certain areas.

The link between the geographical origin, authenticity and traceability of foods is apparent. The EU Council Regulations EC No. 509/2006 and 510/2006 protect certain European agricultural products and foodstuffs as traditional specialities guaranteed and their geographical indications and designations of origin, respectively.\textsuperscript{69,70} For example, saffron (\textit{Crocus sativus} L.)\textsuperscript{71} and buffalo mozzarella\textsuperscript{72} from specific regions in Italy hold the denomination of Protected Designation of Origin (PDO). The hazelnuts (\textit{Corylus avellana} L. cv. 'Tonda Gentile Trilobata') from Piedmont\textsuperscript{63} and the 'Interdonato' lemons of Messina\textsuperscript{73} are example of foods of Protected Geographical Indication (PGI). The Finnish foods under the origin-related EU-regulation include the \textit{Lapin puikula} (almond potato from Lapland), the \textit{Lapin Poron liha} (reindeer meat from Lapland), the \textit{Kainuun rönttönen} (traditional pasty from Kainuu region), the \textit{Kitkan viisas} (vendace from lakes of Koillismaa highlands) and the \textit{Puruveden muikku} (vendace of Lake Puruvesi), holding either the PDO or PGI designation.\textsuperscript{70} In addition, the Finnish beer, \textit{sahti}, the Karelian pasty, \textit{karjalanpiirakka}, and the \textit{kalakukko}, which is fish baked inside a loaf rye bread, are recognised as Traditional Specialities Guaranteed (TSG). The TSG label is granted on the basis of the area and methods of production. However, the control of these Finnish specialities does not include chemical analyses nor are they likely to be subject to fraud other than mislabelling.

\subsection*{2.1.3 Abiotic stress}

In general, stress can have adverse effects on plant growth, development and productivity. Abiotic stress refers to stress caused by exposures to extreme chemical or physical settings, unlike biotic stress, which is triggered generally by a physical injury or pathology caused by another organism. Abiotic environmental stress can negatively impact crop productivity and quality, to the extent that global food security can be compromised. Environmental stress factors include excess/shortage of light, heat or water, nutrient deficit, high edaphic salinity and exposure to phytotoxic substances. Environmental factors may promote oxidative stress that is caused by the formation of reactive oxygen species (ROS; singlet oxygen, hydrogen peroxide, superoxide anion,
hydroxyl radical, perhydroxyl radical). As a result, the plant secondary metabolism activates and antioxidative ascorbic acid, \( \alpha \)-tocopherol, carotenoids and flavonoids are being synthesised. The stress may also induce epigenetic changes. The plant genotype, developmental stage and the type of impacted tissue or organ define how the crops respond to the stress – by resisting or succumbing. The severity and duration of the stress, number of exposures and the effect of other concurrent stressors also define the outcome. Resistance to stress results in survival and improved tolerance through the expression of defence metabolites but susceptibility can lead to death. The plant metabolism is altered as the signal transduction pathways activate after stress recognition. The mitogen-activated protein kinase (MAPK) cascades operate in stress responses and signalling related to hormones and reactive oxidative species (ROS), resulting in changes in downstream signal transduction, metabolism and/or gene expression.\(^{43,74-76}\)

In crop plants, stress can induce changes in assimilation, water and nutrient uptake, secondary metabolism, programmed cell death and gene expression.\(^{75}\) Elevated temperatures are generally stressful to plants and can for example expose crops to yield losses and pest invasion via warm winds.\(^{13}\) Metabolically, heat stress can increase protein levels in cereals and oilseeds.\(^{75}\) High temperatures and radiation can limit the formation of anthocyanins (and colour) in wine grapes,\(^{17}\) carotenoids in carrots and tomatoes,\(^{27,65}\) and lycopene in tomatoes.\(^{27}\) Dry growth season correlates also with lower levels of stilbenes and viniferins in grapes.\(^{17}\) However, heat and/or high-UV stress has been shown to increase the concentration of phenolic antioxidants, ascorbic acid and carotenoids, for example, in apples and lettuce.\(^{75}\) Intense light can induce the biosynthesis and accumulation of protective flavonoids and anthocyanins, whereas shading can alter the composition of anthocyanins and reduce the accumulation of flavonols.\(^{17}\) In cloudberries, cool and rainy growth season induces significantly higher content of anthocyanins and the unsaturation level of fatty acids compared to warm and dry summer that in turn promotes the berry yield.\(^{11}\) Shade can promote the formation of \( \alpha \)-tocopherol and reduce the level of citric acid in cloudberries.\(^{11}\)

The quality and duration of radiation vary in different areas of the world. Light and the phase of light regime encompass irradiance and its spectral composition, polarisation and photoperiod. At northern latitudes, the light regime is at its most extreme, with lengthy periods of either midnight sun or polar nights. High latitudes generally correlate with cooler climates and long photoperiods yet with reduced exposures to solar irradiation, causing a stressful growing environment for plants. The solar angle and the time of day dictate the spectral distribution of red and far-red light.\(^{7,77}\) Light is richer in blue radiation in the north and can promote chlorophyll formation, for example, in spinach
and lettuce.65

Despite its prerequisites, agriculture in northern latitudes is possible as a result of long-lasting acclimatisation and adaptation.13 In general, the northern conditions have been shown to increase the phenolic content of several berries7, like bilberries78 and sea buckthorn10,32. Blackcurrants, on the other hand, exhibit higher levels of phenolics, including flavonols and anthocyanins, when grown in lower latitudes.4,12 Still, high latitude promotes the accumulation of aromatic acids in blackcurrant berries. Plant response to different environments is seen as plasticity. Whereas high temperatures can limit the formation of anthocyanins, low temperatures can induce their accumulation.17 Cold stress can immobilise membrane lipids and slow down reaction kinetics. In long term, cellular damage, dehydration, energy depletion and metabolic dysfunction may occur. Several important food crops, such as potatoes, fruits, berries and vegetables are prone to suffer from night frosts.15 Soil frost and snow cover night frost can delay sowing and seed germination on the spring. Chilling stress can promote the activity of several enzymes, including catalase, glutathione and superoxide dismutase, in several plant species. Temperate and sub-arctic crops can withstand cold stress by increasing the level of membrane phospholipids. Sugars (sucrose, glucose, fructose, raffinose and stachyose), proline and glycinebetaine can accumulate to further advance tolerance to freezing temperatures.76 Bilberries of northern clone was shown to ripen faster than the ones of southern clone when grown in cold temperature by Uleberg et al. Moreover, the content of anthocyanins, total phenolics, malic acid and sucrose was significantly higher in the northern clone.78 Relating to northern conditions, low temperature can especially favour delphinidin glycosides, while long day length can significantly favour the formation of delphinidin, malvidin, peonidin and petunidin glycosides in bilberries. Similarly, high latitude and related conditions correlate positively with flavonol glycosides in sea buckthorn berries.32 Sea buckthorn transcriptome shows promotion of flavonoid, carotenoid and photosynthetic pathways under cold and freeze stress.79

Water deficit can promote the biosynthesis of anthocyanins and stilbenes in wine grapes.17 Water stress can also be caused by freezing temperatures through dehydration and ice crystal formation. Osmotic regulation by accumulating cellular solutes helps stress-tolerant plants to acclimatise to drought or salinity. Such known osmolytes are e.g. proline, glycinebetaine, sugar alcohols and raffinose. Most of these function also as osmoprotectants that are able to scavenge hydroxyl radicals.76 Green tea (Camellia sinensis L.) exposed to high temperatures and precipitation contain more theanine, while cooler areas with low precipitation result in relatively higher levels of amino acids, epi(gallo)catechins, epi(gallo)catechin-3-gallates and caffeine.80 Water
deficiency increases the formation of trans-piceid (trans-resveratrol 3-\(O-\beta-D\)-glucoside), the biosynthesis of stilbene precursors and the expression of stilbene synthase in Cabernet Sauvignon grapes.\textsuperscript{81} Tomatoes elicit genotype-specific responses when subjected to water deficit at different developmental stages.\textsuperscript{82} The water deficit during cell division phase can accumulate more sugars and less acids in one genotype, while deficit during fruit maturation can result in less sugars, acids and carotenoids in the other. Hence, with controlled water deficit at a right developmental stage, tomato genotypes of poor quality can turn out sweeter and tastier, whereas genotypes perceived as of good quality in normal conditions may suffer loss of sensory and nutritional quality.

High altitude can expose plants especially to high ultraviolet (UV) radiation. As a result of depleting ozone layer, exposure to UV-radiation has increased in the polar areas.\textsuperscript{7} The altitude of vineyard can have significant difference in the phenolic metabolites of wine.\textsuperscript{17} As radical scavengers, phenolic compounds can accumulate to protect the plant as flavonoids and anthocyanins can absorb UV-radiation. The levels of stilbenes, for example, tend to increase as altitude increases. Stilbenes, including the health-promoting resveratrol, are products of the phenylpropanoid–acetate pathway.\textsuperscript{76} However, the resveratrol biosynthesis may be under cultivar-specific control.\textsuperscript{81} In sea buckthorn berries, high altitude correlates negatively with sugars and positively with ascorbic acid, malic acid and flavonol glycosides.\textsuperscript{32,67} Sea buckthorn grown in high altitudes has been shown to exhibit higher resistance against drought and UV-B radiation.\textsuperscript{83}

\subsection*{2.1.4 Temporal variation}

Grapes and wine are prime examples of the effect of seasonal variation on the products as the composition of the grape berry is mostly influenced by the vintage (year of harvest).\textsuperscript{17} Seasonal or yearly variation owe to annual fluctuations in weather conditions. Also the within-season variation in temperature, precipitation and winter conditions can severely influence crop growth, maturation, yield, quality, and the timing of agricultural operations.\textsuperscript{13-15} The yearly variation in blackcurrants, according to Vagiri et al., was seen as a significant \((p < 0.05)\) difference among monomeric anthocyanins, flavonols, phenolic acids, ascorbic acid, soluble solids and titratable acidity.\textsuperscript{4} In walnut oil, the content of tocopherols and volatile compounds is influenced by the crop year.\textsuperscript{49} Additionally, the combined effect of variety and crop year is significant for palmitic and linolenic acids.

Growing degree days and season dynamics can vary substantially season-to-season and influence the crop metabolism and ripening.\textsuperscript{13,57} The stage of ripeness, relating to the time of harvest, defines the physical and chemical attributes of food crops. Raffo et al. showed that as sea buckthorn berries gain
weight, the soluble solids and total organic acids decrease.\textsuperscript{84} The level of carotenoids increased towards late harvest. Anesi et al. reported a declining trend of hydroxycinnamic acid derivatives and flavan-3-ols in grapes from \textit{véraison} (start of harvest, early maturity) through mid-harvest to full maturity stage.\textsuperscript{17}

In Italian cherry tomatoes harvested throughout the year, the seasonal variation was shown mostly in the levels of \(\alpha\)-tocopherol and unsaturated lipids, or chlorophylls and phospholipids, depending of the cultivar.\textsuperscript{27} For example, the heat and light conditions during summer repressed the formation of carotenoids (lycopene). The crop age and the phase of the growth season also affect the chemical and sensory properties of green tea shoots.\textsuperscript{85} In a study by Liu et al., the tea leaves collected at early spring season were scored highest over mid- and late spring harvests, outperforming in infusion colour, aroma and taste.\textsuperscript{85} According to Kallio et al., the best harvesting time for sea buckthorn berries in terms of vitamin C and tocopherols/tocotrienols would be late August and early-to-mid September (in southwest Finland), respectively.\textsuperscript{24}

To further narrow down the window for metabolic fluctuation within a timeframe, diurnal variation in temperature and sunlight can also have an impact on plant metabolism and respiration. The photoperiodic cycle (circadian rhythm) under the natural environmental conditions affect the organism’s metabolic system daily.\textsuperscript{86} Short nights at high latitudes reduce the time to transform assimilation products to storage compounds. Relatively high dry matter content can be an indicator of this.\textsuperscript{65} Among certain plants growing in water-starving areas, the crassulacean acid metabolism (CAM) is a photosynthetic carbon fixation pathway and an alternative route for the C\textsubscript{3} and C\textsubscript{4} pathways. CAM-plants, an example of which is the pineapple (\textit{Ananas comosus}), one of the few food crops utilising CAM photosynthesis, undergo large diurnal changes in organic acids and sugars.\textsuperscript{87,88} Especially, the level of malic acid increases nocturnally as a result of carbon assimilation while the sugar levels increase during the day.\textsuperscript{87,88} In herbs, the diurnal fluctuation can be seen in their essential oil content. Spearmint (\textit{Mentha spicata} L.), for example, was shown to produce maximum oil content at 9 a.m., while the optimum time for a high limonene and carvone yield was reached at 9 p.m. and at 3 a.m., respectively.\textsuperscript{28}

\subsection{2.2 Sea buckthorn berries}

Sea buckthorns (\textit{Hippophaë} spp.) are thorny deciduous shrubs of the oleaster family (Elaeagnaceae) that are highly adaptable to changing and stressful environmental conditions like drought, cold and salinity. The most eminent and commercially important species, \textit{Hippophaë rhamnoides} L., has naturally
scattered in different regions of Asia and Europe\textsuperscript{,89,90} In addition to the array of natural species and subspecies, numerous cultivars have been developed for specific properties, such as fruit yield and quality, ease to harvest and resistance to cold, pests and diseases.\textsuperscript{91,92} Sea buckthorn berries are used both traditionally in folk remedies and as raw materials for foods, nutraceuticals and medicines. Common food uses include juices, jams and alcoholic drinks.\textsuperscript{89,92} However, the use of sea buckthorn is not limited to the berries, as the leaves, seeds and bark can be utilised for various pharmaceutical, cosmetic and food applications.\textsuperscript{92} In Finland, the wild sea buckthorn of ssp. \textit{rhamnoides} is spread and thriven along the waterfronts of the Baltic Sea and the Gulf of Bothnia.\textsuperscript{89} The development of sea buckthorn is highly dependent on temperature and light conditions. Vegetation begins when daily average temperature reaches 5–7 °C, while flowering requires temperature of 10–15 °C.\textsuperscript{93}

The berries of sea buckthorn are characterised by their yellow-to-reddish orange colour, spherical-to-cylindrical form of 3–12 mm in length, oleaginous pulp, tangy flavour and high nutritional value.\textsuperscript{89,91} The berries contain a wide variety of nutrients and bioactive compounds, including unsaturated fatty acids, carotenoids, tocopherols, tocotrienols, sterols, phenolic compounds, vitamins, sugars, sugar alcohols, glucose derivatives, inositol, free amino acids and minerals.\textsuperscript{10,92,94-97} \textit{O}-Ethyl \textbeta-\textd-glucopyranoside (henceforth ethyl glucoside; \textbf{Figure 2}) is a compound characteristic to sea buckthorn but rarely found in other fruits. Ethyl glucoside was first elucidated from the sea buckthorn berries in 2006 by Tiitinen, Yang, Haraldsson, Jonsdottir and Kallio.\textsuperscript{98} In 2014, the presence of a corresponding methyl derivative was reported.\textsuperscript{99} The composition of sea buckthorn berries is known to vary due to the influence of their genotype, growth place, harvesting time, agro-climatic parameters and ripening.\textsuperscript{3,24,26,67,84,96,100-102}

\begin{figure}
\centering
\includegraphics[width=0.3\textwidth]{fig2.png}
\caption{\textit{O}-Ethyl \textbeta-\textd-glucopyranoside (ethyl glucoside).}
\end{figure}

Sugars and acids are the main components affecting the sensory properties of sea buckthorn\textsuperscript{103}. The main saccharides in sea buckthorn berries are glucose and fructose, with traces of sucrose.\textsuperscript{100} Sugars are formed and accumulated in plants as the result of the primary metabolism initiated by photosynthesis.\textsuperscript{76} The sugar content in sea buckthorn has been shown to correlate with growth
In general, the berries contain more sugar when grown in non-subarctic conditions and low altitudes.\textsuperscript{3,67} The amount of total sugar in 100 ml juice (ssp. \textit{mongolica}) varies between 1.7 and 10.1 g, most of which is glucose.\textsuperscript{67,100} In the juice of ssp. \textit{sinensis}, the levels vary dramatically depending on the growth location (0.4–24.2 g/100 ml)\textsuperscript{67,100}. In the juice of ssp. \textit{rhamnoides}, the total sugar level is approximately 3 g/100 ml at highest.\textsuperscript{100}

Citric and malic acids are the predominating acids in most fruits. In sea buckthorn, malic and quinic acids dominate, with minor levels of citric acid.\textsuperscript{104} Organic acids function as intermediates in the respiratory metabolism (Krebs cycle) and as storage compounds in vacuoles. The total acidity can decrease during ripening as the acids may be used in respiration or converted to sugars.\textsuperscript{84,100} Acids are important for the taste (sourness) and sensory acceptance of fruits and berries. For example, the sugar/acid ratio correlates with sweetness and pleasantness of sea buckthorn juice.\textsuperscript{103,104} The levels of malic and quinic acid are influenced by the altitude and the latitude of the growth place.\textsuperscript{67} High altitude is also known to increase the level of malic acid.\textsuperscript{67}

Contributing to the overall antioxidative capacity and nutritional value of sea buckthorn berries, vitamin C (ascorbic acid) is another key metabolite in terms of quality and nutritional value. Level of vitamin C in the berries varies between 0.3 and 3.0 g/100 g fresh weight (and 0.03 and 1.70 g/100 ml juice)\textsuperscript{3,24,67,91,95} and is rivalled practically only by rosehip (\textit{Rosa} spp.). Especially, the berries of ssp. \textit{sinensis} are also very rich in vitamin C.\textsuperscript{24} Also, the berries of the Finnish cultivars 'Terhi' and 'Tytti' have a high content of ascorbic acid of up to 2 and 3 g/100 g, respectively.\textsuperscript{91} The ascorbic acid content in the berries is not significantly affected by specific weather conditions\textsuperscript{3} but can decrease during ripening\textsuperscript{24,84}. However, low latitude and high altitude are related to higher levels of ascorbic acid (ssp. \textit{sinensis}).\textsuperscript{67} Yearly variation is generally not significant.\textsuperscript{24,67} Ascorbic acid can deplete at refrigerated temperatures which can reduce the nutritional value of sea buckthorn juice.\textsuperscript{105}

Alkyl glucosides, of which ethyl and methyl glucoside are found in sea buckthorn may have a role as osmolytes. Other reported sources of methyl glucoside are roses (\textit{Rosa hybrid} L.)\textsuperscript{106} and white clover (\textit{Trifolium repens} L.)\textsuperscript{107}. Ethyl glucoside is found in yuzu (\textit{Citrus junos}) peel.\textsuperscript{108} Both compounds can be absorbed in the blood stream and excreted in urine postprandially but their physiological effect, if any, has remained unknown.\textsuperscript{99,109} However, the ethyl glucoside may contribute negatively in the pleasantness of sea buckthorn juice.\textsuperscript{110} The content of ethyl glucoside is wide-ranging among subspecies and cultivars.\textsuperscript{3,100} For example, the berries of ssp. \textit{sinensis} and ssp. \textit{mongolica} contain only traces of ethyl glucoside compared to ssp. \textit{rhamnoides} in which the levels may reach up to 1.9 g/100 ml juice.\textsuperscript{100} In addition, the levels can increase during maturation to the calibre of 2.5–3.0 g/100 ml.\textsuperscript{100}
Inositols and methylinositols are cyclitols that have many biological functions in plants as protective, stress-adaptive metabolites. Myo-inositol and its derivatives, for example, take part in many biosynthetic and regulatory pathways, including carbohydrate and lipid metabolism, promoting plant growth and development. Cyclitols are also important for animal and human physiology. For example, L-quebrachitol may function as gastroprotective and antidiabetic agent when consumed.

In addition to the aqueous metabolites, the origin of sea buckthorn is relevant for the contents of phenolic compounds, lipids and lipid-soluble metabolites. Phytosterols are present in both pulp and seed. Characteristic to sea buckthorn, the soft part is rich in palmitoleic acid (16:1n−7). The berries of ssp. carpathica were shown to contain 53–97 mg/100 g (dw) carotenoids by Pop et al. The berries of ssp. sinensis contain higher levels of flavonol glycosides, most of which are 3-O-glycosides of isorhamnetin and quercetin, compared to ssp. mongolica. Consisting of (epi)gallocatechins as the main monomer units, B-type proanthocyanidins serve as essential contributors to the total antioxidant activity of the fruit. Tocopherol and tocotrienol levels reported by Kallio et al. ranged from 40 mg/kg (fw berry flesh) in ssp. rhamnoides to 120 mg/kg in spp. sinensis. In comparison, values ranging from 22 mg/kg to at 43 mg/kg have been reported in Canadian cultivars.

2.3 Seeds of oilseed rape and turnip rape

Oilseed rape (Brassica napus) and, to a lesser extent, turnip rape (Brassica rapa) are important crops not only for vegetable oil production, but as raw material for biofuel, protein and fibre. In layman’s terms, rapeseed and canola oils may refer to any cooking oil of B. napus ssp. oleifera, B. rapa ssp. oleifera or Brassica juncea. Genome-wise, B. napus and B. rapa represent types AACC (2n = 38) and AA (2n = 20), respectively.

According to FAOSTAT, in 2014, the production quantity of rapeseed was approximately 71 million tons globally. The contribution of Europe and Finland was 29 million (41%) and 62,000 tons (0.09%), respectively. These seed oils are naturally rich in essential polyunsaturated fatty acids, linoleic acid (9,12-octadecadienoic acid; 18:2n−6) and α-linolenic acid (9,12,15-octadecatrienoic acid; 18:3n−3, ALA). Especially, the abundance of ALA results in optimally low ratio of n−6/n−3 fatty acids for human diet. The dietary omega-3 fatty acids have been linked to a reduced risk of cardiovascular and metabolic diseases, stroke and cancer. However, the susceptibility of the double bonds for oxidation during storage, cooking and digestion can again lead to undesirable physiological responses like oxidative stress and inflammation.
Canola and other modern rapeseed cultivars are bred to no longer contain erucic acid (13-docosenoic acid; 22:1n−9), hazardous to human health, and glucosinolates.\textsuperscript{48}

The oil content and quality are determined by the genome of the oil plant and its interactions with the environment.\textsuperscript{125} The genetic regulation is mainly based on the maternal factors. Turnip rape is a freely cross-pollinating heterozygote, while oilseed rape is mainly self-pollinating homozygote. However, when grown in open-air, the oilseed rape can also undergo cross-pollination, yielding in less pure genotypes. Yet, effect of the maternal genotype on the seed oil is considered stronger than the pollen’s.\textsuperscript{125} The oilseeds grown under open-field conditions are susceptible to several biotic and abiotic stresses that can affect the oil composition.\textsuperscript{118}

In \textit{B. napus}, the level of photosynthetic activity in leaves and siliques correlates with the seed yield and the oil quality.\textsuperscript{126} Photosynthetically assimilated carbon is transported into seeds (sinks) mainly as sucrose.\textsuperscript{127} Its glycolysis plays an important role in initiating the seed maturation. Sugar transport in seed coat potentially regulates the oil synthesis by controlling sugar concentration in ovules.\textsuperscript{128} Oilseed rape and turnip rape are considered ripened approximately 114 and 102 days after sowing, respectively.\textsuperscript{129} Stress conditions can delay the seed ripening by a week.\textsuperscript{21}

The synthesis and accumulation of oil starts in the early stage of the embryo development.\textsuperscript{125} The \textit{de novo} biosynthesis of fatty acids and triacylglycerols (TAGs) takes place in the \textit{sn}-glycerol-3-phosphate pathway (Kennedy pathway).\textsuperscript{130} Monounsaturated and saturated fatty acids are synthetised in plastid and transported for a stepwise acylation in the endoplasmic reticulum (ER) by the enzyme fatty acyl elongase (FAE). The key enzymes for the synthesis of polyunsaturated fatty acids are the fatty acid desaturases (FAD2 and FAD3) that target the monounsaturated fatty acyls attached to the \textit{sn}-2 position of phosphatidylcholine. The expression of corresponding genes, \textit{FAD2} and \textit{FAD3}, decreases toward ripening.\textsuperscript{21} The acyl-CoA pool provides fatty acids for the acylation, which is controlled by the acyltransferases GPAT (acyl-CoA:sn-glycerol-3-phosphate) and LPAAT (acyl-CoA:lysophosphatidic acid acyltransferase). The final acylation of a diacylglycerol to a triacylglycerol is catalysed by the acyl-CoA:diacylglycerol acyltransferase (GPAT).\textsuperscript{127} The TAGs are stored in the oil bodies of the mature seed, within the cytoplasm of cotyledon cells. The oil content can reach 50%, w/w. According to Miller et al., flowering is initiated approximately at 580–670 and 470–550 °Cd (when the base temperature is 0 °C) in \textit{B. napus} and \textit{B. rapa}, respectively.\textsuperscript{131} Similarly, the seed filling starts at 970–1070 and 830–930 °Cd, while maturity is reached at 1330–1450 and 1150–1280 °Cd. During the final weeks before maturation, the TAG composition of turnip rape seed oil remains fairly constant.\textsuperscript{132}
Phosphatidylcholine is an alternative intermediate in the TAG biosynthesis. It also serves as an important membrane lipid and acyl carrier. Sinapine, or O-sinapoylcholine, is a characteristic metabolite to Brassicaceae (Figure 3). It is considered to be a supplier of choline for phosphatidyl choline biosynthesis. The esters of sinapic acid are products of the phenylpropanoid pathway. Sinapic acid esters are assumed to be formed via serine carboxypeptidase-like acyltransferases. The metabolic route entails uridine diphosphate (UDP)-glucose:sinapate glucosyltransferase (SGT), sinapoylgucose:choline sinapoyltransferase (SCT), sinapoylgucose:L-malate sinapoyltransferase (SMT) and sinapoylcholine esterase (SCE). According to Boucherau et al., the dry seed of *B. napus* can contain 36 µmol/g of phenolic choline esters, of which 72% is sinapine. In addition to influence of genotype, the content of aromatic cholinyl esters are subject to environmental influence. Intensive light/UV-stress is known to promote the formation of sinapates, as they may function as UV-protectants. Drought, again, lowers the levels of sinapine, especially when encountered during the early vegetative growth period.

![Fig. 3 O-Sinapoylcholine (sinapine).](image)

The antinutritive properties and the unpleasant taste of sinapine and sinapates may limit the use of *Brassica* oilseeds in feedstock. Other antinutrients present in rapeseed meal are glucosinolates (0.5–8%, depending on the cultivar). As secondary metabolites, sinapates and glucosinolates may have a role in plant defence, e.g. against biotic and abiotic stress, like UV radiation and drought. Moreover, rapeseed phenolics have shown potential bioactivity against oxidation and inflammation *in vitro*. As a feed crop, oilseed rape is more applicable due to its higher protein yield per hectare compared to turnip rape. Low oil content may correlate with higher protein content, however, a clear trade-off between protein and oil in *B. napus* and *B. rapa* has not been shown. However, heat stress has been shown to increase protein in *B. napus*. Global warming can reduce the oil content in the seeds of oilseed rape and turnip rape as they have to respond to warmer temperatures during growing season. This may eventually compromise the oilseed
production especially in the northern latitudes. However, the abiotic stress caused by lower temperatures and short day can increase the level of the nutritionally important α-linolenic acid in *B. rapa*.\textsuperscript{21} Water stress can have an effect on rapeseed quality not only by limiting vegetative growth but also by changing seed lipid composition and by accumulating phenolics and glucosinolates.\textsuperscript{137} Water deficiency during vegetative growth can especially decrease the level of oleic acid.\textsuperscript{137} Drought, heat and salt stress can decrease the lipid concentration in oilseed rape.\textsuperscript{75}

2.4 Honey

Honey, as the Council Directive 2001/110/EC\textsuperscript{143}, for example, defines, is the natural sweet food produced by the honeybee, *Apis mellifera*. The bees collect and modify floral nectar or honeydew (excreted by plant-sucking insects, *e.g.* aphids) to be stored and ripened in the honeycomb. The basic components of honey are fructose and glucose. The relative amounts of these monosaccharides, as well as the other saccharides, organic acids and minor components of honey are related to the origin of the honey raw material.

While foraging, the bees pollinate important food crops, like oilseeds, nuts, fruits and berries, increasing the overall meaning and value of beekeeping and honey production. The significance of farmed bees is increasing as the natural pollinators are diminishing. Pollination increases crop yield and quality while improving ripening, shelf-life and resistance against disease.\textsuperscript{144,145}

Blossom, or floral, honeys can be derived from several (multifloral honeys) or mostly from one specific plant source (unifloral or varietal honeys). The botanical origin highly affects the chemical and sensory (flavour, odour, colour, texture) properties of the honey. Whenever a specific origin-related product name is used, it should come from the indicated source (botanical, regional, territorial or topographical) and possess characteristic sensory, physicochemical and microscopic properties.\textsuperscript{143} General criteria for the chemical composition of honey include the content of fructose, glucose and sucrose, moisture, water-insoluble content, electrical conductivity, free acidity, diastase activity and the hydroxymethylfurfural (HMF) content.\textsuperscript{143} Varietal honeys are generally priced higher than regular multifloral or bulk honeys. Varietal honeys may possess unique bioactivities owing to their plant origin. For example, buckwheat (*Fagopyrum esculentum*) honeys have shown promising antioxidant and antimicrobial activities against human pathogenic bacteria.\textsuperscript{146,147}

Harmonised methods for the characterisation and quality control of honeys have been established by the International Honey Commission and are implemented through the Codex Alimentarius standard 12-1981 and the
Council Directive on honey. These include melissopalynological, physicochemical and sensory analyses.

Conventionally, the botanical origin of honey is determined by performing the melissopalynological, sensory and physicochemical analyses, including electrical conductivity, sugars, enzyme activity, proline, colour, optical rotation, pH and acidity. For example, the overall content of fructose and glucose, the fructose/glucose ratio and glucose/water ratio are approximate indicators of botanical origin. Still, known problems with the established methods exist. Firstly, the high natural variability among honeys from different botanical and geographical origins introduces great challenges for the analytical methods. Moreover, the apicultural practices on how to control the bees’ foraging behaviour are limited. Secondly, this extensive set of method is laborious and time-consuming while, to some extent, requiring special expertise. None of the methods give unambiguous result on the actual nectar source. Sensory analysis, usually performed by the beekeepers themselves, is quick and simple but highly prone to subjectivity and the lack of comprehensive proficiency. However, it also allows the evaluation of quality defects, such as fermentation. The pollen analysis, on the other hand, does not guarantee reliable reference to the actual plant source in terms of the collected nectar. Pollen grains in the honey can be carried over with the respective nectar or from exogenous sources. Certain pollen types can be under- (e.g. *Taraxacum* spp.) or over-represented (e.g. *Brassica* spp.) or from non-melliferous sources (e.g. *Filipendula* spp.), distorting the interpretation of the pollen profiles. The annual variation in pollen profiles can be up to 10%. The pollen types found most frequently in Finnish honeys are *Trifolium repens*, *Rubus* spp., *Salix* spp. and Brassicaceae. Honeydew honeys are of non-floral origin, since their raw material is the sugary secretions (honeydew) of plant-sucking insects, such as aphids (*Hemiptera* spp.).

The term ‘unifloral’ is quite often misrepresentative in the case of Finnish honeys having the characteristics of a certain botanical origin. In Finland, bee hives have to be transported to specific locations, e.g. to bogs, in order to collect varietal honeys. Often the Finnish varietal honeys do not meet the given standards, and are therefore placed in an unfavourable situation commercially. Therefore, more specific methods resting on the compositional characteristics of nectar and respective amendments in the regulations permitting their official use is recommended.

The standard physicochemical analyses, although giving accurate data on the honey composition while relating to the nectar source, do not reveal any specific characteristic for different honey types. Therefore, a method which is based on specific botanical markers is required. Several chromatographic and spectroscopic methods have been applied in the analysis of honey phenolics,
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volatiles, amino acids and saccharides that could serve as markers of botanical origin.\textsuperscript{68,151,154-156}

To further challenge for the honey analytics, together with \textit{e.g.} olive oil, milk and saffron, honey is one of the most adulterated foods in the world.\textsuperscript{157} Especially, the manuka (\textit{Leptospermum scoparium}) honey, indigenous to New Zealand, Tasmania and Australia, and the most highly priced and sought-after honey globally, is subject to extensive adulteration and mislabelling. Manuka honey contains methylglyoxal, which is claimed to be the active compound behind the renowned antimicrobial and antiviral properties of the honey. Adulterated honeys usually contain additions of sugar or sugar syrups. The botanical or geographical origin may also be fraudulently declared. Authentication can be performed with \textit{e.g.} chromatography or spectroscopy, by revealing conflicting metabolic profiles.\textsuperscript{155,158-160} The \textit{C}_4-sugars from sugar cane and corn can be detected with GC, HPLC, MS and SNIF-NMR.\textsuperscript{160,161}

2.5 Nuclear magnetic resonance in food and agriculture

2.5.1 Nuclear magnetic resonance (NMR) spectroscopy

The phenomenon of nuclear magnetic resonance (NMR) in condensed matter was first introduced by physicist Bloch and Purcell and their groups in the turn of 1945–1946\textsuperscript{162,163}. Later, following the introduction of Fourier transform (FT) method by Ernst\textsuperscript{164}, multidimensional NMR and cryomagnets, NMR techniques have gained firm foothold not only in structural elucidation of chemical compounds but in the routine, high-throughput applications in physical, chemical and biological sciences, including food sciences.\textsuperscript{165,166}

NMR spectroscopy is based on the magnetic properties of atomic nuclei, \textit{i.e.} nuclei with a non-zero spin quantum number ($I$) will be NMR-active. For example, the proton ($^1\text{H}$) and other common nuclei, carbon-13 ($^{13}\text{C}$), nitrogen-15 ($^{15}\text{N}$), fluorine-19 ($^{19}\text{F}$), silicon-29 ($^{29}\text{Si}$) and phosphorus ($^{31}\text{P}$), have a spin quantum number of $I = \frac{1}{2}$. Nuclei with non-zero quantum numbers act as magnetic dipoles with a magnetic moment $\mu$. The magnetic moment is the product of the gyromagnetic ratio $\gamma$ and the spin quantum number.\textsuperscript{165,166}

When exposed to a strong external magnetic field ($B_0$) the nuclei will be distributed in $(2I + 1)$ energy levels. The small energy difference between the two spin states ($+\frac{1}{2}$ and $-\frac{1}{2}$) depends on the field strength (\textbf{Figure 4}). As the nucleus is irradiated at a nucleus-specific radio frequency, a spin transition occurs. The frequency of this transition is related to the chemical surroundings of the nucleus, hence giving information on how the nucleus is surrounded by neighbouring nuclei. The radio frequency (RF) transmitter detects the absorption of energy and records it as a signal, creating the NMR raw data. The
resulting free induction decay (FID) signal is Fourier-transformed into frequency domain signal. The signals have chemical shifts ($\delta$), expressed as hertz (Hz) or as parts per million (ppm). The $\delta$-values (in ppm) are independent of the operating frequency of the instrument. The chemical shift of a signal is determined by several factors, including electron density, deshielding by the electronegativity of neighbouring atoms, anisotropic effects induced by the magnetic field, deshielding by hydrogen bonding, and affected by variations in temperature, pH, dilution and concentration. The intensity of the signal depends on the differences in the populations of the energy levels. The signal-to-noise (S/N) ratio is proportional to the magnetic field strength, gyromagnetic ratio and number of scans (ns). The acquisition time (aq), relative to the number of data points (td) and spectral width (sw), is typically 1–5 s for proton spectra. Longer AQ improves the digital resolution of fine coupling structures.$^{167}$

![Diagram of nuclei and magnetic field](image)

**Fig. 4** Top: Nuclei in a natural state vs. nuclei subjected to an external magnetic field ($B_0$). Below (left): $\Delta E (= \gamma B_0)$ is the difference in energy between the two energy levels, where the spin can be parallel or anti-parallel to $B_0$. Below (right): Precessional motion of the nuclear magnetic moment, $\mu$, around $B_0$. $^{165,167}$

The $B_0$ causes the electrons in the atom to rotate around their orbitals. The nuclei precess with a frequency $\omega_0$ (Larmor frequency) around the magnetic field (**Figure 4**). As the nucleus returns to the lower energy state, it relaxes. The longitudinal or spin–lattice relaxation ($T_1$) represents the time the nucleus spends at the higher energy state and the energy transfer from the spin to the
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environment of the nucleus (z-magnetisation). The transverse or spin–spin relaxation ($T_2$) relates to the energy transfer between individual spins ($x,y$-magnetisation). The FID fades as the nuclear spins relax back towards equilibrium. The rate of the both relaxation mechanisms is exponential. During the relaxation, the $M_0$ returns to the Boltzmann equilibrium following a spiral pathway. When determining the absolute concentration of a compound, full relaxation is required before applying the next pulse. Hence, the recycling time should be five times the maximum $T_1$. Correction factors may be used when the complete relaxation is not applicable, taking into account the differences in various nuclei and internal standard.\textsuperscript{165,166,168}

The proton exhibits a high natural abundance (99.98\%) and a high relative sensitivity at constant magnetic field.\textsuperscript{165} Carbon-13 of natural abundance of 1.1\%, on the other hand, require significantly longer experimental times to produce adequate data, but is commonly coupled with proton detection in heteronuclear 2D-experiments. $^{13}$C NMR can be used, for example, to determine the relative concentration and the ratio of $sn$-1,3 and $sn$-2 fatty acids in lipidic samples, as Mannina et al. did in the case of sea bass muscle extracts.\textsuperscript{55} The $^{31}$P NMR spectroscopy is worthwhile when targeting e.g. phospholipids in olive oils.\textsuperscript{169} Spyros & Dais have reviewed some of the other food applications of $^{31}$P NMR, including meat and fish (post mortem metabolism), milk (inorganic phosphate, casein-bonded phosphoserine, polyphosphates, phospholipids, casein), starch (starch phosphate monoester, phospholipids, inorganic phosphate), phytate, lecithins, phosphoproteins, oligo- and polyphosphates and organophosphorus pesticides.\textsuperscript{170} Mattinen et al., on the other hand, used quantitative $^{31}$P NMR to determine structural characteristics of suberin polymers in potato skins.\textsuperscript{171}

High-resolution NMR requires a liquid sample. Deuterated solvents are used to provide a deuterium NMR signal for magnetic field stabilisation and to allow optimised resolution for each sample. Most commonly, D$_2$O, DMSO-$d_6$, CD$_3$OD and CDCl$_3$ are used for different applications, respectively. Presaturation can be performed by applying continuous weak RF irradiation at the solvent frequency prior to excitation and acquisition.\textsuperscript{167} The solvent spins are subsequently rendered, leaving them unobservable. A suitable internal standard compound, e.g. TMS (tetramethylsilane) for organic solvents and TSP (3-(trimethylsilyl)propionic-2,2,3,3-$d_4$ acid) or DSS (4,4-dimethyl-4-silapentane-1-sulfonic acid(-$d_6$)) for aqueous solutions, is used to calibrate the chemical shift while serving as a reference for quantitation and a resolution indicator. Volatility (TMS), pH sensitivity (TSP, DSS), protein binding capacity (TSP) and hygroscopicity (DMSO-$d_6$), however, can limit the appropriateness of the substance in quantitative analysis.\textsuperscript{172} In addition to use of purposeful reference compound, the recycle time (d1) and the number of scans...
(ns) are the key parameters to optimise for accurate quantification.

The resonance frequency of a nucleus is dependent on its magnetic environment, namely the neighbouring nuclei (the molecular structure). This is the basis for the NMR’s unique capacity in determining molecular structures. Other factors, such as small deviations in field stability and homogeneity, temperature, pH and solute interactions can cause variation in the chemical shifts. The proton signal splits based on \( n \) number of equivalent neighbouring protons. The splitting pattern of a proton follows the \( n + 1 \) rule, where \( n \) is the number of protons in the adjacent carbon(s). The relative intensity ratios of the split signals and the peak multiplicities comply with the rules of the Pascal’s triangle. The split lines are at equidistance corresponding to the coupling constants between the coupled nuclei. The inter-proton coupling is typically largest, 2–15 Hz, between geminal protons (H–C–H). In vicinal (H–C–C–H) coupling, the coupling constant is determined by the Karplus curve according to the dihedral angle (0–180°) between the protons and can be 0–15 Hz. If the coupling path goes over four or more bonds, the coupling constant is reduced close to zero. Also, coupling over a double bond is subject to the influence of \( \text{cis}/\text{trans} \)-configuration of the protons. Exceptions to the coupling rules are based on molecular symmetry, chirality and equivalency.

An example of a common workflow in elucidation of molecular structures could comprise of \( ^1\text{H}, ^{13}\text{C}, ^1\text{H}–^1\text{H} \) COSY (correlation spectroscopy; proton \( J \)-coupling over 2–3 bonds), TOCSY (relayed proton \( J \)-couplings within a spin system), \( ^1\text{H}–^{13}\text{C} \) HSQC (heteronuclear coupling over one bond), \( ^1\text{H}–^{13}\text{C} \) HMBC (long-range heteronuclear couplings over 2–3 bonds) and NOESY (nuclear Overhauser effect spectroscopy; correlations through space) experiments. The area ratio of integral values of signals serves as an indicator of the relative number of nuclei representing each signal (\(^1\text{H}\)).

As an example of solid-state NMR, the high-resolution magic-angle spinning (HR-MAS) NMR can be applied to the analysis of biological tissues and different food matrices. Powdered or sliced food samples can be analysed as such without time-consuming sample pre-treatment phase. This technique facilitates high-resolution solid-state experiments by eliminating perturbing dipolar coupling and allowing narrow line-widths with the “magic” angle (\( \theta = 54.7° \)) spinning. For example, the quality and metabolic characteristics of semi-solid food samples like capiscum peppers, garlic, lemon juice, mangoes, mozzarella cheese and tomatoes have successfully been studied with HR-MAS NMR. The HR-MAS technique allows deuterium locking. Therefore, the sample is flushed/topped with (saline or buffered) \( \text{D}_2\text{O} \). \(^{13}\text{C} \) cross-polarization (CP)-MAS NMR has been used for dry solid samples, e.g. in structural characterisation of cutin and suberin polymers in fruits, berries, vegetables, cereal and potatoes. The cross-
polarisation improves the S/N of the non-sensitive $^{13}$C nucleus.

Time-domain nuclear magnetic resonance (TD-NMR) is a low-resolution method that measures differences in relaxation properties.\textsuperscript{179} Applicable in food authentication (e.g. milk\textsuperscript{180}) and quality control (beef\textsuperscript{181}), the benchtop TD-NMR instruments, or minispecs, are routinely used in the industry as a standard method in determining fat and water content in food and feed.\textsuperscript{179,182}

The Carr–Purcell–Meiboom–Gill (CPMG) pulse sequence is widely used with biofluids where high-molecular weight metabolites (e.g. proteins) display broader signals than smaller molecules as they have a shorter $T_2$ relaxation time.\textsuperscript{167} As a so-called spin-echo method, it minimises diffusion effects and exchange processes by applying a $90^\circ$ pulse followed by a $(\tau - 180^\circ - \tau_n)$ pulse train.\textsuperscript{165,166} The CPMG pulse can be applied in two-dimensional $T_1$–$T_2$ correlation relaxometry of complex food matrices, like egg white and yolks, fruit parenchyma and hydrocolloids.\textsuperscript{183}

The general principle of site-specific nuclear isotope fractionation (SNIF-) NMR is to measure natural isotope ratios $^{2}$H/$^1$H (or D/H) and $^{13}$C/$^{12}$C. The ratios are influenced by many factors, such as origin and processing.\textsuperscript{184} SNIF-NMR is applied, for example, in the authentication and characterisation of beer\textsuperscript{185}, fruit juices\textsuperscript{45}, honey\textsuperscript{161}, maple syrup\textsuperscript{186}, vanillin\textsuperscript{187} and wine\textsuperscript{45,188}. C$_4$ syrup additions starting at 9–10% can be detected from honey.\textsuperscript{161} As a comparison, chromatographic methods can detect syrup additions of 5–10%.\textsuperscript{160}

The emergence of cryogenic probes (cryoprobes) has increased the sensitivity of NMR instruments as they reduce thermal noise and increase S/N. The RF coils and preamplifiers are cooled while the immediate surroundings of the sample remain at room temperature. Microcoil probes improve again sensitivity and allow the sample volumes to be reduced to a few micro- or nanoliters. Flow-probes are used in the hyphenated hybrid technique of LC–NMR(–MS).\textsuperscript{166} The LC–NMR(–MS) can further enhance the elucidation of natural products and complex plant materials.\textsuperscript{189,190} NMR spectroscopy may revolutionise in the near future, as the use of dynamic nuclear polarisation (DNP) method based on polarisation transfer from electron to nuclei becomes more common. DNP-NMR improves on signal intensities drastically and thus reduces data collection time significantly.\textsuperscript{191}

### 2.5.2 Data pre-processing

The raw NMR metabolomics data usually consists of tens of thousands of data points representing NMR spectra and tens or hundreds of objects representing samples. The NMR data is also susceptible to the effects of the water signal and the variation in pH, temperature and ionic strength.\textsuperscript{37,192} To simplify and optimise the data analysis phase, certain data pre-processing methods need to
be applied. Izquierdo-García et al.\textsuperscript{193} have reviewed some of the common software packages for NMR data processing, including the AMIX (Bruker BioSpin, Germany), matNMR\textsuperscript{194}, Chenomx NMR Suite (Chenomx Inc., Canada), KnowItAll (Bio-Rad Laboratories Inc., CA) and MestReNova (Mestrelab Research, Spain). These packages provide a number of functions for spectral processing and for the identification and quantification of metabolites.\textsuperscript{193} Additionally, the MVAPACK\textsuperscript{195} and the MetaboAnalyst\textsuperscript{196}, for example, provide a platform for statistical and multivariate analysis.

The data processing workflow generally consists of zero filling prior to phase and baseline correction (manual/automatic) and may be complimented by apodization, spectral alignment, peak picking, integration, deconvolution, binning, normalisation and scaling.\textsuperscript{41,197} First, however, the FIDs (time-domain data) are transformed into spectra (frequency-domain data) by applying Fourier transform (FT). In phase correction, a constant or frequency independent parameter (zero-order) and a linear parameter dependent on the frequency (first-order) are added to the spectrum in order to eliminate phase distortions.\textsuperscript{193} The phase correction can be performed manually or with automated algorithms. Baseline correction removes distortions in the baseline that can negatively affect statistical and quantitative analysis.\textsuperscript{198} Automatic baseline correction usually deploys polynomial subtraction and manual corrections can further be made with polynomial, exponential, sine or spline functions.\textsuperscript{193} Baseline fitting can also be based on, for example, automated algorithm with manual point-wise correction\textsuperscript{199}, locally weighted scatterplot smoothing (LOWESS)\textsuperscript{200} or asymmetric least-squares method.\textsuperscript{193,198}

The S/N and the resolution of the spectra can be improved by multiplying the time-domain data prior to FT with a window function (apodisation). Exponential window function improves S/N, the extent of which can be adjusted with line-broadening (LB). Digital resolution can be enhanced with Gaussian window function or zero-filling, if the increase of acquisition time is not desirable. The Gaussian window function improves lineshapes and resolution but also increases noise. In zero-filling, a set of zero data points are added to the FID tail. In the actual spectrum, the zero points are situated between the original data points. However, whenever resolution is improved the S/N is reduced and \textit{vice versa}.

The inhomogeneity of the magnetic field can severely hamper spectral quality with lineshape distortions. Shim correction based on reference deconvolution can be used to eliminate signal non-symmetry and to standardise the spectra for further analyses.\textsuperscript{201}

The NMR peak misalignments along the frequency axis cause unwanted bias and noise in the data analysis phase. The misalignments common in biological samples are usually derived from variance in pH and intermolecular
interactions. While binning may dissipate some of the misalignment, spectral resolution is lost. Examples of peak alignment algorithms include, for example, interval correlated shifting (icoshift), correlation optimised warping (COW), dynamic time warping (DTW), fuzzy warping (FW), hierarchical cluster-based peak alignment (CluPA) and peak or recursive alignment with fast Fourier transform (PFFT/RFFT). Warping algorithms apply local stretching or compression, may form artefacts, are usually computationally complex and thus require hours of execution time. The use of FFT cross-correlation engine speeds up the processing. For example, the icoshift alignment tool, including a FFT function, allows a fast alignment of NMR peaks simultaneously for the whole dataset without forming spectral artefacts.

Signal integration is used to determine the number of nuclei responsible for each signal. Signal integrals, relative to the number of nuclei, are proportional to the molar concentration of the compound and can also be used to determine the absolute concentrations of compounds. Suitable signal-to-noise ratio and narrow lineshapes being the requirements, the duration of recycle time, the number of scans and the receiver gain are critical parameters in achieving a obtain reliable and accurate results. In mixture analyses, signal overlapping, however, can severely complicate the integration and the quantitative analysis. Instead of the peak area, the peak height can also be used in the calculations.

The study by del Campo et al. demonstrated how the NMR quantification of citric and malic acid, commonly present in fruits, is highly affected by the sample pH and the presence of structurally similar aspartic acid. Here, the accurate quantification of these acids from fruit juices required a pH-adjustment to pH 1.0 in order to avoid signal overlapping. Spraul et al. integrated the overlapped components of citric and malic acid multiplets in fruit juices after applying 2D J-resolved experiments that simplified and separated the signals. The accurate integration of overlapping signals can also be obtained with deconvolution, featured for example in the Chenomx NMR Suite. Modelled spectrum (reference compound libraries) and the known concentration of the internal standard are used in the quantification.

Monakhova et al. exploited a set of independent component analysis (ICA) algorithms to further enhance the deconvolution of complex food samples with highly overlapping components such as sugars.

The integration can also be challenged by the water signal. The signal tails, or moreover, the water suppression procedures can interfere the resonances near the water signal. The experimental parameters related to the solvent suppression need to be optimised to eliminate the co-suppressive (saturation) effect in neighbouring signals.

Binning, or bucketing, is a technique often applied in metabolomics when
the size (and unwanted variation) of the dataset is wished to be reduced. In binning, the data is divided into regions and the subsequent region integrals form the reduced dataset in text (and subsequently tabular) form. As the spectral data usually consists of thousands of data points, the number of variables can now be reduced to hundreds to facilitate data analysis. Equally-sized regions of width 0.01–0.04 ppm are often used in metabolomics (rectangular bucketing), while variable-sized custom bins and advanced binning (based on picked peaks) are also possible.\textsuperscript{37,193,212}

The unwanted vertical and horizontal variation can be eliminated from the data (now in tabular form) with normalisation and scaling, respectively. Data normalisation makes the data from all samples directly comparable with each other by generally minimising dilution effects as the variation in concentrations is removed.\textsuperscript{193,213,214} The selected normalisation method can have a significant effect on the outcome of the data analysis.\textsuperscript{214} The data can normalised either to total spectrum area (%) or to the area of a reference compound, highest peak.

Scaling targets the systematic bias in spectral intensities and the often largely varying numerical ranges of variables. Unit variance (UV) scaling divided by standard deviation, giving the variables an equal weight. UV-scaling can therefore create unwanted noise as the low-intensity variables and the baseline can be accentuated while the strong variables are attenuated. However, UV-scaling can be useful when the data consists of variables that have different units, e.g. in multi-platform datasets. Mean-centring by mean subtraction, often accompanied by the UV-scaling (hence auto-scaling), sets all variable means to zero to improve interpretation. In Pareto scaling, the variable is divided by the square root of the standard deviation. Pareto scaling is often the scaling method of choice in NMR metabolomics data as it acts as an intermediate between UV-scaling and mean-centring (also referred to as “no scaling”) and has emphasis on the medium and small features in the data. The variance in Pareto scaling equals the square root of the variable standard deviation. Both normalisation and scaling are imperative for metabolomics-based data analysis.\textsuperscript{213,215}

\subsection*{2.5.3 Multivariate data analysis}

Multivariate data analysis is used to extracting meaningful information from complex metabolomics data. The basic focus areas in multivariate analysis are data overview, pattern recognition, finding similarities/dissimilarities, classification, discrimination, regression and prediction modelling.\textsuperscript{215,216} Principal component analysis (PCA)\textsuperscript{217,218} is the staple in multivariate data analysis. As an unsupervised multivariate projection method, it is used to extract and display systemic variation in the data matrix ($X$), consisting of rows (observations) and columns (variables). PCA gives an overview of the data,
revealing trends, pattern, groups and outliers. Principal components (PCs) are linear combinations of the original variables and describe the variation in the dataset in descending order of magnitude. The score matrix of a PCA model represents the observations as coordinates relative to the PCs. A scatter plot of the score vectors ($t$) provides a summary or overview of all observations (or samples) in the data table. Loadings matrix describes which variables contributed in to the respective PCs. The loading vectors ($p$) define the relation among the measured variables. A loadings plot shows the influence or weight of the individual $X$-variables in the model and gives information of the basic patterns within the data. A model’s goodness-of-fit (explained variation) and predictive ability are expressed as $R^2$ and $Q^2$, respectively.\textsuperscript{166,215,219}

Hierarchical cluster analysis (HCA), also an unsupervised method, is used to classify and cluster observations and/or variables based on their similarities and dissimilarities. Classified as either agglomerative or divisive HCA, the clusters are paired from bottom up or from top down in hierarchy, respectively. As a result, a cluster tree (dendrogram) is formed. The distance between clusters indicates the (dis)similarity.\textsuperscript{166,215} For example, Mazzei et al. used agglomerative HCA to classify mozzarella cheese examined with $^1$H HR-MAS NMR and applied Euclidean distances to measure the similarity between sample groups.\textsuperscript{72} As a result, two major clusters were formed according to the geographical origin of the cheeses.

Supervised multivariate, or pattern recognition methods are characterised by the inclusion of the data matrix $Y$, representing dependent variables. Partial least squares (PLS) projections to latent structures is a regression method used to find linear relationships between data blocks $X$ and $Y$ (containing quantitative values).\textsuperscript{220} PLS is commonly used in discriminant analysis (PLS-DA), the $Y$ now consisting of qualitative values. Discriminant analysis requires homogenous classes in order to work. The binary code is applied to set class memberships as dummy variables forming the $Y$ matrix. Although powerful methods as such, the PLS-based models may be negatively affected by the non-related systemic variation between the matrices.\textsuperscript{166,215,219} The orthogonal partial least squares (OPLS) is an adaptation of the PLS that separates the systemic variation in $X$ into that of linearly related to $Y$ and of unrelated, or orthogonal, to $Y$. Again, discriminant analysis can be applied (OPLS-DA). As the $X$-variation is concentrated to only one predictive component with one $Y$, and the non-predictive variation is filtered to orthogonal components, the model interpretability is improved. However, several orthogonal components can exist. The orthogonal variation allows the examination of the within-class variation. Bidirectional OPLS (O2PLS) is predictive towards both $X$ and $Y$, modelling joint $X$–$Y$ covariation and the $Y$-orthogonal variation in $X$ or vice versa. Despite their predictive performance, both PLS and OPLS models can
easily be overfitted and their predictability overestimated under certain conditions, for example, in the occurrence of non-homogenous distribution of observations in classes and excess interclass variation.\textsuperscript{215,219,221}

Model validation is a fundamental part of the multivariate data analysis, ruling out chance and substantiating the significance of the model. Predictive models can be validated by cross-validation, permutation test, external validation, and by predicting new observations. Cross-validation, or internal validation, gives estimates of the significance of a latent variable and the general predictive power of the model. In the cross-validation procedure, the data is divided into $K$ groups followed by model formations, excluding one group every time. For example, a sevenfold cross-validation is applied in the SIMCA-P+ by default. In a permutation test, the position of $Y$-data is randomly shifted (e.g. 20 times) to appear in different order. The permuted values are compared to the $R^2Y$ and $Q^2Y$ estimates of the original model. As a result of the permutation test, the $Y$-axis intercepts of $R^2$ and $Q^2$ in a regression line plot should be less than 0.3–0.4 and 0.05, respectively, to indicate model validity.\textsuperscript{215,222}

External validation is considered a comprehensive validation method that consists of building a training set and a test set with independent observations. The training set must be able to predict for example the class membership of the samples in the test set and any new observations.\textsuperscript{215,222}

Besides PLS-based supervised multivariate methods, are linear discriminant analysis (LDA), $k$-nearest neighbours ($k$NN), soft independent modelling for class analogy (SIMCA) and artificial neural networks (ANN) among those of frequently utilised with NMR spectroscopic and metabolomics data. For example, Santos et al. applied SIMCA and $k$NN in the classification of authentic and adulterated milk samples analysed with $^1$H TD-NMR.\textsuperscript{180} Both models exhibited generally good sensitivity and specificity, although some false positives were revealed.

### 2.5.4 Metabolomics

Metabolomics is a field of research concentrating on studying the metabolites within a biological entity at a given time. The metabolome represents a complex of compounds derived from dynamic and complex biochemical processes of primary and secondary metabolism occurring in tissues, cells, organs or organisms, in interaction with the environment.\textsuperscript{31} Food metabolome can be defined as the pool of compounds present, subject to the influence of food origin, processing and storage.\textsuperscript{223} As food is generally of plant or animal origin, metabolomics of biological systems parallels to food metabolomics.\textsuperscript{37} The vast diversity of close to 200,000 metabolites within plants makes plant
foods much more complex metabolically than animal foods. Metabolomics, as so aptly put by Ward and others (2007), “... can not only assist in a deeper understanding of the complex interactive nature of ... metabolic networks and their responses to genetic change but also will provide unique insights into the fundamental nature of ... phenotypes in relation to development, physiology and environment”. Metabolomics is a powerful tool in functional genomics, plant systems biology and agriculture as it can provide a universal overview of plants combining phenotypic, morphological, clinical and other biological data with DNA, RNA, protein and metabolite analyses. Agricultural applications include trait development and biorefining. For example, omics-based phenotyping strategies are used in rapeseed breeding. Along with genomics, proteomics and transcriptomics, metabolomics complements the omics tools.

NMR metabolomics explores metabolic events and outcomes by extracting meaningful information from complex spectroscopic data with chemometrics. Non-NMR approaches for metabolomics study include HPLC, LC–MS, GC–MS, CE–MS (capillary electrophoresis–mass spectrometry), FT-IR (Fourier transform infrared spectroscopy) and FT-ICR–MS (Fourier transform ion cyclotron–mass spectrometry). The key advantages of NMR over chromatographic and mass spectrometric methods include simple preparation without derivatisation (e.g. silylation in GC) or fractionation, basically full recovery of samples, relatively fast analysis, simultaneous detection of several compound classes of varying concentrations and high reproducibility. In addition, NMR is quantitative and yields very little solvent waste. However, costly instrumentation and maintenance and low sensitivity (although, today, to a lesser extent) are recognised disadvantages of NMR. Even though the metabolomics approach with NMR allows the holistic and comprehensive analysis of the sample composition, only a mere fraction of the whole metabolome can be assessed at one time, as the dynamic range of metabolites can vary from picomolar to molar.

Closely related to metabolomics terminologically and ideologically, metabonomics is focused on drug metabolism and systems biology research on biofluids. The term foodomics, referring to the use omics-techniques in field of food chemistry and nutrition, was first introduced in 2009 by Alejandro Cifuentes and subsequently adopted by researchers applying either NMR or mass spectrometry in food metabolomics. Metabolic fingerprinting is a global screening method suitable for complex samples as it involves the comprehensive and simultaneous analysis of a wide variety of metabolites, omitting the quantification of individual metabolites. Metabolic profiling experiments follow a more limited set of metabolites often through specific pathways and can involve metabolite identification and
As a useful tool for screening biological systems and dynamic processes, metabolic profiling can be used to identify changes in major metabolites according to plant genotypes.\cite{41,234} However, this approach is less applicable in identifying minor differences between sample groups, or variances in metabolites present in low levels.\cite{234} Targeted profiling focuses, according to Weljie et al.\cite{210}, on quantitative analysis of specific metabolites.\cite{35} Untargeted approach in metabolomics, on the contrary, refers to the general exploration of the sample composition without specific target metabolites or the need for metabolite identification and quantification.

The general NMR metabolomics workflow, as illustrated in Figure 5, include sample preparation for direct analysis or for analysis after extraction and data acquisition, processing, analysis and interpretation.\cite{37,41} The biological variation (time of harvest, developmental stage, enzymatic degradation, oxidation) of foodstuffs should be taken into account in experimental design, sampling and sample conservation in order to avoid rapid metabolic changes.\cite{37,225,228,235} Flash-freezing using liquid nitrogen, freeze-drying and storing at \(-80\) °C are preferred methods to retain the sample integrity in the post-sampling phase.\cite{37,228,235} Lyophilisation can also be used as a method for sample concentration and for minimising the intensity of the water resonance in the proton spectra.\cite{37} With liquid foodstuffs such as juices, wine, oils and honey, a direct analysis with only a small addition (\(\geq 5\% - 10\%\)) of \(d\)-solvent (including internal standard) combined with a solvent-suppression sequence is applicable.\cite{37,228} Otherwise, an extraction step is required. The choice of extraction solvent predefines the metabolites in focus. For example, Sobolev et al. analysed both aqueous (D\(_2\)O) and lipidic fractions (CD\(_3\)OD/CDCl\(_3\), 3:2, v/v) of lettuce leaves for a more comprehensive metabolic coverage of sugars, amino acids, phenolic compounds, carotenoids, pheophytins, sterols, several classes of lipids and hydrocarbons.\cite{236} Typical sample volumes of 300–750 µl may be cut down to a few microliters when so called micro- or nanoprobe are used.\cite{235} pH-adjustment by using a buffer solution or manually with additions of NaOH and HCl are usually applied to minimise variation in chemical shifts.\cite{37,228} Procedures for metabolomics, including sample preparation, have been thoroughly reviewed for example by Beckonert et al.\cite{233} and Mannina et al.\cite{37}

In the past decade, NMR metabolomics in plant and food analysis has advanced substantially. The applications in the analysis of food composition, quality and authenticity have already covered most food categories: fruits and berries,\cite{29,51,237-240} vegetables,\cite{18,22,27,29,175,177} fish,\cite{30,55,56,241} meat,\cite{54,242-245} cheese,\cite{72,246-248} milk,\cite{180,249-251} honey,\cite{59,252-257} vegetable oils,\cite{169,258-262} juice,\cite{73,209,263-265} tea,\cite{60,266} coffee,\cite{267-270} wine,\cite{62,188,271-273} spirits,\cite{274} cocoa,\cite{64} spices,\cite{71,275,276} as previously reviewed.\cite{37,40} In addition, metabolomics has been
Fig. 5 Schematic illustration of the metabolomics workflow in food analysis.
harnessed to accommodate research on food consumption and physiological monitoring in nutritional interventions.\textsuperscript{35} \textbf{Table 1} summarizes the most recent NMR metabolomics studies on foodstuffs published during 2010–2016.

In agriculture, metabolomics can improve the consistency, predictability and cost-effectiveness in plant breeding when producing quality food resistant to stress and with high nutritional value.\textsuperscript{277} Discrimination of anomalous metabolic profiles can be applied in detecting of crop pathologies such as tomato mosaic virus\textsuperscript{278} and citrus greening disease\textsuperscript{279}, or genetically modified crops\textsuperscript{280,281}. Similarly, organically produced foods, potatoes\textsuperscript{282}, tomatoes\textsuperscript{22} and milk\textsuperscript{251} as examples here, can be authenticated based on their metabolic response to the farming system, specified by e.g. the type of fertilisation used and the subsequent availability of nutrients such as nitrogen. Kim et al., Romero et al. and Sanchéz Peréz et al. used NMR metabolomics to study the effect of maturation or ripening on blackberries\textsuperscript{238}, olive oil\textsuperscript{283} and tomatoes\textsuperscript{18}, respectively.

As with the naturally occurring biochemical changes in the food metabolic pathways pre-harvest, the changes occurring during food processing and storage are equally ideal subjects for metabolomics-type investigation and monitoring.\textsuperscript{223} NMR metabolomics is especially trending in monitoring fermentation, as it allows a rapid screening of metabolic patterns during the dynamic process, with the inherent prospect of identifying novel compounds. Piras et al. studied metabolic changes due to the use of carbohydrates, acid production and proteolysis during the natural ripening process of cheese.\textsuperscript{247} Also, the adjunct cultures had an effect on e.g. citric acid levels.\textsuperscript{247} Spevacek et al. monitored beer metabolites in different brewing conditions.\textsuperscript{284} Dry hopping was shown to alter the yeast purine metabolism during fermentation, producing significantly different levels of adenine, adenosine and 2'-deoxyadenosine compared to late hopping.\textsuperscript{284} The extent of tea fermentation (oxidation) process can be seen, for example, in the levels of flavan-3-ols, gallic acid and caffeine, as shown by Lee et al.\textsuperscript{80}

Santucci et al. monitored the occurring in climacteric fruits (peaches, plums and tomatoes) during post-harvest cold storage.\textsuperscript{29} Castejón et al. used PCA and PLS regression to classify meat samples according to their storage time and to predict the ageing time, respectively.\textsuperscript{244} The \textsuperscript{1}H NMR data of meat exudates were used to build the models. To monitor fruit freshness and shelf-life, Capitani et al. used the $T_2$ spin–spin relaxation time-based measurements to determine the water status of intact kiwifruits\textsuperscript{237} and blueberries\textsuperscript{285}. The water status analysis with a portable instrument is fast and, together with metabolite profiling and targeted analysis, can give a comprehensive result of the commercial grade and overall quality of the foodstuff.\textsuperscript{237,285} Rochfort et al. combined flavour-, aroma- and mouthfeel-related parameters from sensory
Table 1 Recent NMR metabolomics-related research on different foodstuffs (2010–2016).

<table>
<thead>
<tr>
<th>Food sample</th>
<th>Solvent</th>
<th>MHz</th>
<th>Data analysis</th>
<th>Subject</th>
<th>Key metabolites</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Beef</td>
<td>D$_2$O</td>
<td>600</td>
<td>PCA, OPLS-DA</td>
<td>Discrimination of geographical origin</td>
<td>Amino acids, succinic acid</td>
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<td>500</td>
<td>PCA, PLS</td>
<td>Effect of conservation and ageing</td>
<td>Fatty acids, acetic acid, amino acids, sugars, nucleotides, inosine</td>
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<td></td>
<td>CDCl$_3$</td>
<td>60,</td>
<td>PCA</td>
<td>Authentication (beef vs. horse meat)</td>
<td>Lipids</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>600</td>
<td></td>
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<td></td>
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<tr>
<td></td>
<td>D$_2$O</td>
<td>600</td>
<td>PCA, CT</td>
<td>Metabolic profiling, classification according to the level of irradiation</td>
<td>Glycerol, lactic acid esters, $p$-substituted phenolic compound</td>
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<td>Beer</td>
<td>10% D$_2$O</td>
<td>500</td>
<td>PCA, PLS-DA</td>
<td>Ageing</td>
<td>Organic acids, dextrins, aromatic compounds</td>
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<td></td>
<td>10% D$_2$O</td>
<td>600</td>
<td>PCA, LMM</td>
<td>Compositional changes during brewing processes, effect of hopping method</td>
<td>Sugars, amino acids and derivatives, nucleotides and derivatives, energy- and fatty acid metabolism-related metabolites, vitamins</td>
<td>284</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(late-hopped vs. dry-hopped)</td>
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<td>Black raspberry</td>
<td>D$_2$O; CD$_2$OD/</td>
<td>600</td>
<td>PCA, PLS-DA</td>
<td>Effect of maturation stage</td>
<td>Sugars, amino acids, organic acids, phenolic compounds</td>
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<tr>
<td>(Rubus coreanus)</td>
<td>D$_2$O/TFA-d; CDOD$_3$</td>
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<td>Bovine milk</td>
<td>D$_2$O</td>
<td>600</td>
<td>PCA</td>
<td>Classification, effect of breed and coagulation properties</td>
<td>Carnitine, lactose, citric acid, choline</td>
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<td></td>
<td>CDCl$_3$</td>
<td>400,</td>
<td>LDA, FDA, PLS-</td>
<td>Authentication (organic vs. conventional)</td>
<td>Lipids</td>
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<td>DA</td>
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<td>Cabbage (Brassica</td>
<td>CD$_3$OD / sodium</td>
<td>600</td>
<td>PCA, HCA</td>
<td>Discrimination of cultivars and geographical origins</td>
<td>GABA, acetic acid, amino acids, $\alpha$-phosphocholine, phenylacetic acid, succinic acid, sucrose</td>
<td>287</td>
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<tr>
<td>spp.)</td>
<td>phosphate buffer / D$_2$O</td>
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<td>Carrot (Daucus</td>
<td>CDCl$_3$, D$_2$O</td>
<td>600</td>
<td>Multiblock PCA</td>
<td>Effect of genotype</td>
<td>Sugars, amino acids, nucleotides, fatty acids, sterols, $\beta$-carotene</td>
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<td>carota L.)</td>
<td>D$_2$O/CD$_2$OD (2:1),</td>
<td>400</td>
<td>PCA, ANOVA</td>
<td>Effect of genotype, geographical origin and pedoclimatic</td>
<td>Amino acids, organic acids, sugars, sterols, fatty acids, choline, quercetin glycoside, catechin, uridine phosphate, niacinamide, falcarinol, carotenoids</td>
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<tr>
<td></td>
<td>CDCl$_3$</td>
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<tr>
<td>Cheese</td>
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<td>400</td>
<td>PCA, HCA, DA</td>
<td>Quality control, authentication</td>
<td>PUFAs, isobutylic alcohol, lactic acid, acetic acid</td>
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<td></td>
<td>CDCl$_3$</td>
<td>400</td>
<td>PCA</td>
<td>Detection of vegetable fat, discrimination of cheese types</td>
<td>Lipids</td>
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<td></td>
<td>D$_2$O</td>
<td>400</td>
<td>PCA, PLS</td>
<td>Effect of cheese ripening</td>
<td>Amino acids, organic acids, choline, phospho-</td>
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<td>MHz</td>
<td>Data analysis</td>
<td>Subject</td>
<td>Key metabolites</td>
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<tr>
<td>Cherries (Prunus avium L.)</td>
<td>Na$_2$C$_2$O$_4$ buffer</td>
<td>400</td>
<td>PCA, LDA, PLS-DA, SIMCA</td>
<td>Authentication</td>
<td>choline, carbohydrates</td>
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<td>Citrus fruit (Citrus spp.)</td>
<td>10% D$_2$O</td>
<td>600</td>
<td>PCA, OPLS-DA</td>
<td>Characterisation of geographical origin</td>
<td>Malic acid, glucose, fructose, glutamine, succinic acid</td>
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<td>Buffered D$_2$O</td>
<td>400</td>
<td>PLS, cross-validation</td>
<td>The effect of microenvironment</td>
<td>Amino acids, succinic acid, GABA, sugars, limonin glucoside</td>
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<td>KH$_2$PO$_4$ in D$_2$O</td>
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<td>PCR, PLS</td>
<td>Authentication of orange juice</td>
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<td>Cocoa beans (Theobroma cacao L.)</td>
<td>D$_2$O:CDOD$_3$ (8:2)</td>
<td>600</td>
<td>OSC, PLS, PCA, OPLS-DA</td>
<td>Discrimination of species, variety and</td>
<td>Amino acids, organic acids, sugars, caffeine, caffeic acid, epicatechin</td>
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<td>Coffee (Coffea spp.)</td>
<td>Sodium phosphate buffer / D$_2$O</td>
<td>500</td>
<td>PCA, OPLS-DA</td>
<td>geographical origin</td>
<td>Sucreose, caffeine, chlorogenic acids, choline, amino acids, organic acids, trigonelline</td>
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<td>Buffer</td>
<td>500</td>
<td>PCA, OPLS-DA</td>
<td>Authentication and quality of coffee blends</td>
<td>Acetic acid, trigonelline, formic acid, caffeine</td>
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<td>Cola beverage</td>
<td>CD$_3$OD–phosphate sodium buffer</td>
<td>500</td>
<td>PCA, OPLS-DA</td>
<td>Quality assessment, effect of geographical origin</td>
<td>Sucreose, GABA, quinic acid, choline, acetic acid, fatty acids</td>
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<td>(H$_2$O/D$_2$O, 1:1) D$_2$O</td>
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<td>PCA</td>
<td>Quality control</td>
<td>Caffeine, acesulfame-K, aspartame, cyclamate, benzoic acid, HMF, E 150D, vanilin</td>
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<td>Crab meat</td>
<td>Potassium phosphate buffer in D$_2$O</td>
<td>400</td>
<td>PCA, PLS-DA</td>
<td>Discrimination of species, nutritional</td>
<td>Glutamic acid, alanine, glycine, homarine, lactic acid, betaine, taurine, MUFAs</td>
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<td>DCD$_1$</td>
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<td></td>
<td>characterisation, quality assessment</td>
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<td>Crab paste</td>
<td>K$_2$HPO$_4$/NaH$_2$PO$_4$ in H$_2$O/D$_2$O</td>
<td>400</td>
<td>PCA, OPLS-DA</td>
<td>Quality, effect of fermentation time</td>
<td>Lactic acid, betaine, taurine, trimethylamine-N-oxide, trigonelline, inosine, adenosine diphosphate, 2-pyridinemethanol, amino acids, glutamic acid, sucreose, formic acid, acetic acid, trimethylamine, hypoxanthine Amino acids, organic acids, fatty acids, glucose, organosulphur compounds, allicin</td>
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<td>Garlic (Allium sativum L.)</td>
<td></td>
<td>400</td>
<td>PLS-DA</td>
<td>Classification of cultivars and geographical</td>
<td></td>
<td>173</td>
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<td>origins</td>
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<td>MHz</td>
<td>Data analysis</td>
<td>Subject</td>
<td>Key metabolites</td>
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<td>Grape (Vitis spp.)</td>
<td>DMSO-d6/D2O</td>
<td>600</td>
<td>PCA</td>
<td>Effect of thermal processing</td>
<td>Fructose, glucose, acetic acid, formic acid, pyrogulatamic acid, cycloallii, HMF</td>
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<td>Grape marc spirit</td>
<td>CD3OD : KH2PO4 buffer in D2O (1:1)</td>
<td>600</td>
<td>PCA, PLS-DA, OPLS-DA</td>
<td>Metabolic characterisation of cultivars</td>
<td>Amino acids, choline, sugars, organic acids, flavonoids, phenylpropanoids</td>
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<td>Honey</td>
<td>D2O : oxalate buffer</td>
<td>600</td>
<td>PCA, OPLS-DA</td>
<td>Effect of origin, grape genotype and vintage</td>
<td>Amino acids, ethyl esters, fusel alcohols, succinic acid, lactic acid, sugars, polyols</td>
<td>274</td>
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<tr>
<td>Hazelnut (Corylus avellana L.)</td>
<td>CD2OD</td>
<td>600</td>
<td>PCA</td>
<td>Authentication, effect of origin, classification (raw vs roasted)</td>
<td>Trigonelline, amino acids, o-disubstituted aromatic compound</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>CDCl3</td>
<td>600</td>
<td>PCA, PLS-DA</td>
<td>Authentication, effect of origin, classification (raw vs roasted)</td>
<td>Terpene acids, hexanal, chrysin, pyrrolodine derivative</td>
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<tr>
<td></td>
<td>D2O</td>
<td>400</td>
<td>PCA, HCA, KNN, SIMCA, PLS-DA</td>
<td>Classification of botanical origin, discrimination and detection of adulterations, prediction of honey type</td>
<td>Amino acids, sugars, organic acids, HMF, ethanol</td>
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<td></td>
<td>CDCl3</td>
<td>600</td>
<td>PCA, O2PLS-DA</td>
<td>Discrimination of botanical origin</td>
<td>Flavonoids, γ-LACT-3-PKA, B-hydroxylinalool, dehydromofoliol, caffeine, monoterpenes</td>
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<td>Human breast milk</td>
<td>D2O</td>
<td>500</td>
<td>OPLS-DA</td>
<td>Discrimination of geographical origin</td>
<td>Mono-, di-, tri- and tetrasaccharides</td>
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<td>10% D2O</td>
<td></td>
<td>OPLS-DA</td>
<td>Discrimination of botanical origin</td>
<td>Formic acid, tyrosine, phenylacetic acid, dehydromofoliol</td>
<td>253</td>
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<td>KH2PO4 in D2O / H2O / D2O</td>
<td>400</td>
<td>ICA, JADE</td>
<td>Authentication, determination of botanical origin, quantification of metabolites</td>
<td>Sugars, organic acids, HMF, ethanol, methylglyoxal, dihydroxyacetone</td>
<td>159</td>
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<tr>
<td>Ice cream</td>
<td>D2O phosphate buffer</td>
<td>400</td>
<td>PLS-DA</td>
<td>Discrimination (secretors vs. non-secretors)</td>
<td>Fucose, 2'-fucosyllactose, 3'-fucosyllactose lactotrifucotetraose, lacto-N-tetraose, lacto-N-fucopentaoase I, 6'-sialyllactose 2'-fucosyllactose, lactotrifucotetraose, lacto-N-fucopentaoase, lacto-N-difucohexaoeses</td>
<td>296</td>
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<tr>
<td>Mango (Mangifera spp.)</td>
<td>CDCl3 Oxalate buffer / D2O</td>
<td>400</td>
<td>PCA</td>
<td>Detection of vegetable fat</td>
<td>Fatty acids and esters</td>
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<td></td>
<td></td>
<td>500</td>
<td>PCA</td>
<td>Discrimination of cultivars</td>
<td>Arginine, histidine, phenylalanine, glutamine, shikimic acid, trigonelline</td>
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<td>Food sample</td>
<td>Solvent</td>
<td>MHz</td>
<td>Data analysis</td>
<td>Subject</td>
<td>Key metabolites</td>
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<tr>
<td>Mussels</td>
<td>D$_2$O; CDCl$_3$</td>
<td>800</td>
<td>PCA, PLS-DA</td>
<td>Discrimination of species, effect of origin</td>
<td>Glucose, amino acids, homarine, organic acids, taurine, betaine</td>
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<tr>
<td>Olive oil</td>
<td>CDCl$_3$</td>
<td>500</td>
<td>PCA, LDA, PLS-DA, SIMCA, CART</td>
<td>Authentication, geographical characterisation</td>
<td>Alcohols, sterols, hydrocarbons, tocopherols</td>
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<td>Food sample</td>
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<td>MHz</td>
<td>Data analysis</td>
<td>Subject</td>
<td>Key metabolites</td>
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<td>301</td>
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<td>PCA, OPLS-DA</td>
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<td>Effect of fermentation</td>
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<td>EDTA in $D_2O$</td>
<td>400</td>
<td>PCA, LDA</td>
<td>Authentication (organic vs. conventional)</td>
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<td>PCA</td>
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<td>177</td>
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<td>$CDCl_3$</td>
<td>400</td>
<td>PCA</td>
<td>Effect of growing season</td>
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<td>27</td>
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<td>$Na_2HPO_4/NaH_2PO_4$ in $D_2O$</td>
<td>600</td>
<td>PCA, PLS</td>
<td>Effect of post-harvest storage</td>
<td>Amino acids, ferulic acid, succinic acid, galacturonic acid, sucrose, UDPG</td>
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<td>Tomato (Solanum lycopersicum L.)</td>
<td>$H_2O/D_2O$</td>
<td>500</td>
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<td>Theanine, isoleucine, leucine, valine, alanine, threonine, glutamine, quinic acid, glucose, epicatechin, epigallocatechin, epigallocatechin-3-gallate, caffeine</td>
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<td>EDTA in $D_2O$</td>
<td>400</td>
<td>PCA</td>
<td>Tissue differentiation, effect of ripening</td>
<td>Sugars, cutin, malic acid, citric acid, lipids, amino acids</td>
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<td></td>
<td>$D_2O$</td>
<td>700</td>
<td>PCA</td>
<td>Authentication (organic vs. conventional)</td>
<td>Malic acid, asparagine, aspartic acid, fructose, glucose, histidine, choline, threonine, trigonelline, adenosine monophosphate</td>
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<tr>
<td></td>
<td>$CDCl_3$</td>
<td>400</td>
<td>PCA</td>
<td>Effect of growing season</td>
<td>α-Tocopherol, unsaturated lipids, chlorophylls, phospholipids</td>
<td>27</td>
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<td>$Na_2HPO_4/NaH_2PO_4$ in $D_2O$</td>
<td>600</td>
<td>PCA, PLS</td>
<td>Effect of post-harvest storage</td>
<td>Amino acids, ferulic acid, succinic acid, galacturonic acid, sucrose, UDPG</td>
<td>29</td>
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<tr>
<td></td>
<td>$H_2O/D_2O$</td>
<td>500</td>
<td>PCA</td>
<td>Effect of geographical origin</td>
<td>Theanine, isoleucine, leucine, valine, alanine, threonine, glutamine, quinic acid, glucose, epicatechin, epigallocatechin, epigallocatechin-3-gallate, caffeine</td>
<td>266</td>
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<tr>
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<td>EDTA in $D_2O$</td>
<td>400</td>
<td>PCA</td>
<td>Tissue differentiation, effect of ripening</td>
<td>Sugars, cutin, malic acid, citric acid, lipids, amino acids</td>
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<td></td>
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<td>Authentication (organic vs. conventional)</td>
<td>Malic acid, asparagine, aspartic acid, fructose, glucose, histidine, choline, threonine, trigonelline, adenosine monophosphate</td>
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<td>400</td>
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<td>Effect of growing season</td>
<td>α-Tocopherol, unsaturated lipids, chlorophylls, phospholipids</td>
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<td>$Na_2HPO_4/NaH_2PO_4$ in $D_2O$</td>
<td>600</td>
<td>PCA, PLS</td>
<td>Effect of post-harvest storage</td>
<td>Amino acids, ferulic acid, succinic acid, galacturonic acid, sucrose, UDPG</td>
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<td>Food sample</td>
<td>Solvent</td>
<td>MHz</td>
<td>Data analysis</td>
<td>Subject</td>
<td>Key metabolites</td>
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<td>CD&lt;sub&gt;3&lt;/sub&gt;OD</td>
<td>500</td>
<td>PCA, PLS, OPLS, O2PLS</td>
<td>shading Sensomics, effect of wine type and vintage</td>
<td>succinic acid, malic acid, glycerol, ethanol, 2,3-Butanediol, malic acid, proline, tartaric acid, lactic acid, threonine, flavonols, flavan-3-ols</td>
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<tr>
<td>10% D&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>600</td>
<td>PCA, ECVA, iECVA, PCA, LDA, MANOVA</td>
<td>Effect of fermentation stage, production year, origin (terroir) Authentication; effect of variety, geographical origin and vintage</td>
<td>Sugars, ethanol, lactic acid, malic acid, isopentanol, isobutanol, Shikimic acid, caftaric acid, 2,3-butanediol, lactic acid, acetic acid, proline, succinic acid, malic acid, glycerol, tartaric acid, glucose, phenolic compounds, amino acids</td>
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<td>KH&lt;sub&gt;2&lt;/sub&gt;PO&lt;sub&gt;4&lt;/sub&gt; in D&lt;sub&gt;2&lt;/sub&gt;O</td>
<td>400</td>
<td>PCA, ECVA, iECVA, PCA, LDA, MANOVA</td>
<td>Authentication; effect of variety, geographical origin and vintage</td>
<td></td>
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</tbody>
</table>

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a HR-MAS, high-resonance magic-angle spinning.  
b 13C-based analysis.  
c Quantitative in situ analysis. Abbreviations: ANN, artificial neural network; ANOVA, analysis of variance; CART, classification and regression tree; CT, classification tree; DA, discriminant analysis; DModX, distance to model; HCA, hierarchical cluster analysis; ICA, independent component analysis; (i)ECVA, (interval) extended canonical variate analysis; JADE, joint approximate diagonalisation of eigenmatrices; K-index, indicator of fish freshness; KNN, k-nearest neighbours; LDA, linear discriminant analysis; LMM, linear mixed-effects model; MANOVA, multivariate analysis of variance; O(2)PLS(-DA), orthogonal partial least squares (discriminant analysis); OCS, orthogonal signal correction; OCPLS2, orthogonal constrained partial least squares projection to latent structures; OPLS-W2A, orthogonal Wold’s two-blocks Mode A PLS; PCA, principal component analysis; PLS, partial least squares / projection to latent structures; PLS-DA, partial least squares discriminant analysis; pt-PLS, post-transformation PLS; SIMCA, soft independent modelling of class analogy.
analysis with NMR metabolomics data to evaluate the quality characteristics and sensory properties of wines.\textsuperscript{271} The rapid and inexpensive analysis can reduce the need for sensory analysis in wine quality control.\textsuperscript{271} The high-throughput, automated, push-button NMR-based analytical tools have taken field in food industry as they improve the cost-efficiency and the time management of food quality control. For example, the quantitative flow-injection NMR with automated data analysis is used in mixture analysis of fruit juices, as described by Spraul et al.\textsuperscript{209}

The use of non-targeted fingerprinting in official food control was reviewed by Esslinger et al.\textsuperscript{302} The quality assessment and authentication of virgin olive oil by NMR spectroscopy was reviewed by Dais & Hatzakis.\textsuperscript{169} Although highly applicable in food authentication and detection of hazards or manipulations, food fingerprinting is challenged by appropriate validation, standardisation, harmonisation and databases in order to function as a routine procedure.\textsuperscript{40,302}

\subsection*{2.6 Summary}

Food, as it ultimately consists of tissues, cells, organs or organisms of animal, plant and/or microbial origin, and of the products of their metabolism, is susceptible to numerous endo- and exogenous factors influencing its composition and quality. The origin of a food or a raw ingredient is one of the key elements that influence the occurrence and concentration of food metabolites. The sensory properties, shelf life and the nutritional quality of foods are subject to extensive variation derived from the genetic foundation, growth place, weather, soil, agricultural practices, harvest, processing and storage conditions.

The food genotype (species, subspecies, variety, cultivar and breed) determines the preconditions but adaptation and acclimation to varying environments increase the prevalence of different phenotypes by altering the primary and secondary metabolism. To a large extent, the geographical origin determines the climatic characteristics and prevailing abiotic stress factors, mainly on account of latitude and altitude. High latitudes generally correlate with cooler climates and long photoperiods but reduced exposures to solar irradiation. The abiotic stress can result in a higher bioactive and nutritional value of the food plants as they aim to protect themselves by accumulating secondary metabolites. The natural fluctuations in weather conditions cause seasonal variation between harvests. For example, the sea buckthorn berry composition is subject not only to genetic variation among subspecies and cultivars. As highly adaptable to harsh environments, the berries of sea buckthorn exhibit different phenotypes according to the growth conditions. The
time of harvest and the stage of ripeness also contribute to the berry composition and quality. Similarly, the *Brassica* oilseeds, naturally rich in linoleic acid and $\alpha$-linolenic acid, show compositional variation according to their genetic and geographical background. Abiotic stress can delay the seed development and ripening and alter the lipid, protein and secondary metabolism.

Honey, as an example of food susceptible for origin-related fraudulence, is a multifaceted natural product representing various floral sources and/or honeydew of insect origin. The compositional, sensory and physical characteristics of honey reflect the source of nectar (or honeydew) collected and processed by the honeybees. The limitations of the established practices in honey quality control can lead to ambiguity in the honey characterisation and to subsequent mislabelling. Both the botanical and geographical origins of honey are targets for economically motivated adulteration and fraud.

NMR spectroscopy, especially when used in metabolomics, offers a wide aptitude in food analysis aiming to advance food authenticity, traceability, quality and safety and to understand the biological mechanisms derived from the origin-related prerequisites. Metabolomics is an effective tool in understanding metabolic pathways through non-targeted and targeted analysis of metabolites associated with specific genotypes, geographical origin, exposure and/or resistance to biotic and abiotic stress, and seasonal variation related to the food origin and prevailing growth conditions. Chemometrics and multivariate tools facilitate the analysis of complex mixtures that foods generally are and enable the relevant information to be extracted and interpreted. The advantage in the fundamentally untargeted character of NMR metabolomics allows novel findings, such as bioindicators of environmental stress or markers of origin, to surface.
Aims of the Study

3 AIMS OF THE STUDY

The general aim of the study was to use NMR metabolomics to investigate effect of origin on the composition and quality of sea buckthorn berries, Brassica oilseeds and honey. With sea buckthorn berries and oilseeds, the objective was to investigate the effect of genotype and growth environment, with special emphasis on the effect of northern latitudes and related conditions. With honey, the objective was to authenticate the botanical origin and the key markers of Finnish honeys.

The objectives of the individual studies were to:
I Investigate the effect of genotype and growth environment on wild sea buckthorn berries of two subspecies from Finland and China, respectively;
II Investigate the effect of genotype, growth environment and the developmental stage on the seeds of oilseed rape and turnip rape in Finland;
III Discriminate and find markers of Finnish varietal honeys of different botanical origins; and
IV Investigate the effect of genotype and growth environment on sea buckthorn berries of two Finnish cultivars grown in different locations in Finland and Canada.
4 MATERIALS AND METHODS

4.1 Samples

The sample sets in this study were (I) wild sea buckthorn berries (*Hippophaë rhamnoides* ssp. *rhamnoides* from Finland and *H. rhamnoides* ssp. *sinensis* from China), (II) ripened and developing seeds of oilseed rape (*Brassica napus*) and turnip rape (*B. rapa*) from five locations in Finland, (III) honeys of nine different botanical origins and (IV) cultivated sea buckthorn berries (*H. rhamnoides* ssp. *rhamnoides* cv. 'Terhi' and 'Tyttri') from different growth sites in Finland and Canada.

4.1.1 Sea buckthorn berries

4.1.1.1 Wild sea buckthorn

Two of the sub-studies were focused on sea buckthorn berries, *Hippophaë rhamnoides* L. (Elaegnaceae). In the first study (I), wild sea buckthorn (*H. rhamnoides* ssp. *rhamnoides*;) berry samples were collected from three locations in Finland: Uusikaupunki in 2007–2010 (latitude 61° N, longitude 21° E; altitude 1 m), Kemi in 2008–2009 (65° N, 24° E; 1 m) and Taapajärvi in 2007–2010 (67° N, 24° E; 170 m) (Figure 6). The wild shrubs were transplanted in 1992 from the island of Karta on the coastal area of the Gulf of Bothnia to both Uusikaupunki and Taapajärvi in order to investigate the effect of latitude and environmental conditions on the berry composition. Karta is located 1 km apart from the Uusikaupunki growth site. The berries from Kemi were picked from wild bushes on a small uninhabited island off the coast.

All the Chinese berries (*H. rhamnoides* ssp. *sinensis*) were collected from natural growth sites in six locations in 2006: Hebei (latitude 41° N, longitude 116° E; altitude 818 m; harvest date Oct 29); Heilongjiang (47° N, 127° E; 210 m; Nov 28); Ordos (39° N, 109° E; 1480 m; Nov 24); Shanxi (37° N, 113° E; 1512 and 2182 m; Oct 21); Sichuan (31° N, 106° E; 2000, 2500 and 3000 m; Oct 20) and Qinghai (36° N, 101° E; 3115 m; Oct 29) (Figure 6).

Neither fertilisers nor pesticides/herbicides were used on the bushes. The berries were picked in a randomised manner from several shrubs when optimally ripe (except in Taapajärvi, 2008, when the berries were picked as unripe and semi-ripe), then frozen and stored immediately at −20 °C until analysed. The optimal ripeness for harvest and consumption of the berries was determined by experienced local pickers.
Fig. 6 Suggestive map of the harvest locations of the sea buckthorn berries in Finland and China. Reprinted from the original publication (supplementary), with permission from Elsevier.

4.1.1.2 Cultivated sea buckthorn

In the second sea buckthorn berry study (IV), the focus was on two cultivars of *H. rhamnoides* ssp. *rhamnoides*, 'Terhi' (botanical registry code TTA-361) and 'Tytti' (TTA-362). The berries were hand-picked from cultivation sites in Turku (Sammalmäki), South of Finland (latitude 60°23′N, longitude 22°09′E, altitude 1 m), Kittilä, North of Finland (68°02′N, 24°37′E, 210 m) and Québec, Canada (46°47′N, 71°17′W, 100 m) in four consecutive years of 2007–2010 (from Kittilä 2009 and 2010 only). The bushes were planted in Turku and Kittilä in 2005. For Québec, the one-year old shrubs were translocated bare-root in May 2003, stored in pots and re-planted in June 2004. Again, the berry samples were picked when optimally ripe, frozen and stored at –20 °C until analysis.
Materials and Methods

4.1.2 Brassica oilseeds

4.1.2.1 Ripened seeds

Ripened seeds of low erucic acid spring rape (oilseed rape; *Brassica napus* L. ssp. *oleifera*) and spring turnip rape (*Brassica rapa* L. ssp. *oleifera*) of the 2011 crops were received via MTT Agrifood Research Finland (current Natural Resources Institute Finland – Luke) from the official variety test trial sites in Hauho, Inkoo, Jokioinen, Maaninka and Pernaja (Figure 7).\(^{129}\) The five trial sites situated in different cultivation zones according to their geographical location: Inkoo (I; 60° N, 25° E) and Pernaja (P; 60° N, 26° E) were located in zone I, Jokioinen (J; 61° N, 24° E) and Hauho (H; 61°N, 25° E) in zone II and Maaninka (M; 63° N, 27° E) in zone III, respectively. The zonal classification is based on the temperature sum accumulation of a region during growing season\(^{129}\).

![Figure 7](https://example.com/figure7.png)

**Fig. 7** Locations of the open field sites.\(^{129}\) Reprinted from the original publication\(^{304}\) (supplementary), with permission from Elsevier.

The oilseed rape genotypes were 'Belinda' (hybrid; from I/P/H), 'Brando' (hybrid; H), 'DLE 1006' (I/P/H), 'DLE 1107' (I/P/H), 'Early Bird' (I), 'Highlight' (I/P/H), 'Lunedie' (I), 'Majong' (hybrid; I/P/J/H), 'Marie' (I/P/H), 'Mirco CL' (hybrid; I/J/H), 'Proximo' (I/P/J/H), 'SW Q2865' (I/P/J/H), 'Tamarin' (I/H) and 'Trapper' (hybrid; I/P/J/H). The turnip rape genotypes chosen for this study were 'Aurea CL' (I/P/J/H), 'Bor 05075' (I/P/J/H/M), 'Bor 05100' (I/P/J/H/M), 'Bor 07010' (I/P/J/H), 'Cordelia' (J), 'Juliet' (J), 'SW Petita' (I/P/J/H/M) and 'Viikki 11' (I/P/J/H). Weeds and pests were controlled according to the protocol of the test sites. The plots were harvested when fully matured and the yield
obtained was dried after harvest to a moisture content of approximately 9%. Seeds from one or two block samples were randomly selected for extraction.

4.1.2.2 Developing seeds

The seed development was followed under controlled conditions, representing both optimal and stress conditions. The oilseed rape genotypes 'Marie' and 'Bor 01000' and the turnip rape genotypes 'SW Petita' and 'Bor 05075' were cultivated as described by Vuorinen et al. (2014). Optimal growing conditions were created in a growth room and in a greenhouse at +22 °C with 16 h day length and at +15–20 °C with 16–19 h day length, respectively. Stress conditions were created with the reduced temperature (+15 °C, 16 h) in a growth chamber. The siliques were harvested at and pooled to correspond 2, 3 and 4 weeks after flowering (WAF; start of flowering observed approximately 30 and 50 days after germination for turnip rape and oilseed rape, respectively), with ± 3 days marginal per weekly time point and stored temporarily at −20 °C. The seeds were gently plucked with tweezers (on ice) and stored at −80 °C.

4.1.3 Honey

For the third (III) sub-study, twenty Finnish honeys of varying origins of nectar (or honeydew) were acquired with the aid of the Finnish Beekeepers’ Association (Helsinki, Finland). The honeys were harvested in 2012 and 2013. The botanical origins of the honey samples were buckwheat (*Fagopyrum esculentum*; samples 1, 2 and 20), dandelion (*Taraxacum officinale*; samples 3–5 and 18), heather (*Calluna vulgaris*; samples 10–12 and 19), Himalayan balsam (*Impatiens glandulifera*; sample 14), linden (*Tilia* spp.; sample 9), lingonberry (*Vaccinium vitis-idaea*; sample 16) and clover (*Trifolium repens/hybridum*; sample 8). Three samples of honeydew honeys (samples 6, 7 and 13) and two multifloral honeys (samples 15 and 17) were also included in the study. Samples 1–17 were acquired directly from the producers (of which 3–16 via the Finnish Beekeepers’ Association), while samples 18–20 were bought from a producer’s market stand. The samples were from eleven different beekeepers and 15 geographical origins from different parts of Finland (the exact origins of the samples 18–20 was not known, but they were from the same producer). The honeys were harvested by the beekeepers prior to the end of flowering of the focal plant. Dandelion and lingonberry honeys were harvested at the end of June. All the other honey types, excluding heather, were harvested during late July to early August. Heather honey, as a late variety, was harvested in mid-August. The honeys were handled and stored according to each beekeeper’s customary practices. The samples were stored in the ambient temperature and protected from light prior to analyses.
4.2 Methods

4.2.1 Sample preparation

In studies I and IV, the sea buckthorn berries were deseeded, freeze-dried, and ground in liquid nitrogen prior to extraction. Berry powder (100 mg) was mixed with 4 ml acetone–water (7:3, v/v), vortexed and sonicated for 10 min in RT, followed by centrifugation (Heraeus Labofuge 200, Hanau, Germany) at 968 × g for 10 min. The extraction was repeated after supernatant collection. The pooled supernatants were frozen at −80 °C, after which the samples were dried in a vacuum centrifuge (Heto Holten Maxi-Dry Plus, Allerød, Denmark). The dry residue was dissolved in 800 µl of formate-buffered deuterium oxide (D₂O) containing 0.05% (TSP), pH 3.75. After filtration (0.45 µm), the sample pH was adjusted to 2.70 with 1 N NaOH (in D₂O) and 57% HCl (in H₂O). An aliquot of 600 µl was transferred into a 5-mm NMR tube for analysis.

In study II, the ripened seeds of oilseed rape / turnip rape (100 mg) were extracted in 1.5 ml cyclohexane using an Ultra-Turrax T 25 homogeniser (IKA Works, Wilmington, NC), equipped with an 8 G dispersing element. The extract was centrifuged (1730 × g, 10 min), separated, evaporated under a N₂-flow and re-dissolved in 600 µl chloroform-d (CDCl₃). The seeds were re-extracted with CDCl₃ and methanol-d₄ (CD₃OD), sonicating for 30 min in 1.5 ml solvent, respectively. All three extracts were filtered (0.45 µm) and stored in −20/−80 °C until analysis, prior to which 600 µl of extracts were transferred to 5-mm NMR tubes.

The developing oilseeds were removed from the siliques and pooled according to the time point. The seeds (50 mg) were extracted with 800 µl CDCl₃ by crushing the seeds with a stirring rod, followed by vortexing for 1 min and sonicating for 10 min. The extract was filtered and stored at −80 °C.

In study III, a 100 mg of honey (mixed by stirring) was combined with 800 µl of ultrapure H₂O (Millipore, Billerica, MI). After a 5 min vortexing, the sample was centrifuged (14,000 × g, 1 min, 20 °C) and filtered by ultracentrifugation (3 kDa; Amicon, Millipore). Internal standard DSS-d₆ (23 µl; 4.6187 mM in D₂O, with 0.2% NaN₃) was added to the filtrate (207 µl). The pH of the mixture was adjusted to 6.55 ± 0.05 with NaOH and/or HCl. An aliquot of 180 µl was transferred to a 3-mm NMR tube for analysis. Selected honey samples were also extracted with chloroform and analysed in 600 µl CDCl₃ according to Schievano et al.²⁹⁵

In each study, three independent technical replicates were performed in order to take the effect of potential matrix inhomogeneity into account. Each replicate was analysed once.
4.2.3 Nuclear magnetic resonance (NMR) spectroscopy

The instrument used in studies I, II and IV was a 500 MHz Bruker Avance spectrometer (Bruker BioSpin AG, Fällanden, Switzerland), equipped with a broadband inverse autotune probe (BBI-5mm-Zgrad-ATM) and operating at 500.13 MHz for proton and at 125.76 MHz for carbon-13 (Instrument Centre, Department of Chemistry, University of Turku, Finland). The instrument used in the study III was a 600 MHz Bruker Avance III spectrometer, equipped with a 5-mm triple resonance inverse cryoprobe (CPTCI) and a temperature-controlled SampleJet sample changer (Nuclear Magnetic Resonance Facility, UC Davis, CA).

In studies I and IV, the water-suppressed proton spectra were acquired at 25 °C with 1D NOESY presaturation pulse programme (noesypr1d), consisting of 320 scans, an acquisition time of 3.28 s, a recycle delay of 4 s, a mixing time of 100 ms and a 90°-pulse of 6.9 µs. Free induction decays (FIDs) were collected with 64 k data points, covering a spectral width of 10 kHz. Prior to Fourier transform, an exponential line-broadening function of 0.3 Hz was applied to the FIDs. Other 1D and 2D standard experiments, like DQF-COSY (cosygpmfqf), HMBC (hmbcgplpndqf), HSQC (hsqcetgpsi2), CH2-edited HSQC (hsqcedetgpsisp2), NOESY (noesygp) and 1D TOCSY (selmlgp) were applied to a selection of samples.

In study II, a standard zg30 pulse programme of 160 scans (64 k, 10 kHz) was applied with acquisition time of 3.28 s, relaxation delay of 7 s, and 90° pulse of 6.90 µs. Automatic receiver gain, with the exception of set value of 90 for CD3OD extracts, was used. A selection of samples were subjected to additional DQF-COSY (cosygpmfqf), HMBC (hmbcgplpndqf), HSQC (hsqcetgpsi2), CH2-edited HSQC (hsqcedetgpsisp2) and 1D TOCSY (selmlgp) experiments.

In study III, a noesypr1d pulse programme with 128 scans, 16 dummy scans, 32 k data points, sweep width of 12 ppm, acquisition time of 2.5 s, relaxation delay of 2.5 s, mixing time of 100 ms and receiver gain of 32 was used for the aqueous samples (298 K). An irradiation of 70 dB for water presaturation was applied. The chloroform extracts were analysed with a zg30 pulse at 298 K using 512 scans, 8 dummy scans, 32 k data points and spectral width of 14 ppm.

4.2.4 Spectral processing

The spectra (studies I, II and IV) were pre-processed with TopSpin 1.3 software (Bruker Biospin GmbH, Rheinstetten, Germany), followed by binning with the AMIX toolkit (Bruker Biospin GmbH). In study III, Chenomx NMR
Suite 8.0 (Chenomx Inc., Edmonton, Canada) was applied in both pre-processing and binning. Baseline and phase correction were performed manually and/or automatically. Bin widths of 0.002 (I, IV), 0.04 (II) and 0.02/customised ppm (III) were used. In general, the spectral areas covering solvent peaks and redundant or no information were excluded from the datasets.

In study I, the bin integrals and spectral intensities were scaled to positive intensities and normalised to the reference region of TSP, respectively. The spectral alignment tool icoshift was used in the Matlab environment (MATLAB® R2015b, MathWorks Inc., Natick, MA via CSC – IT Center for Science Ltd.). Customised intervals were designed for the spectral data to minimise spectral misalignment. Correlation shifting based on the calculated average spectrum with additional pre-correlation shifting phase was applied. The maximum allowed shift per interval was determined automatically and the missing values were replaced with ‘NaN’.

In study II, bin widths of 0.04 ppm were used. Scaling to positive intensities and normalisation to total intensity was applied to bin integrals and spectral intensities, respectively. In study III, the spectra were first zero-filled to 128 k data points. Shim correction to 0.5 Hz with subsequent line broadening of 0.5 Hz was applied (Chenomx Processor). The data was normalised to the total spectral area.

In study IV, a shim correction to a linewidth of 0.9 Hz was applied (Chenomx Processor). The data was firstly reduced to 0.02 ppm-sized bins and normalised to total spectral area after which the spectral data from technical replicates were averaged and customised variable-sized bins, containing preferably only signals from single metabolites when applicable, were created in Excel.

The NMR peaks and metabolites were assigned and identified, using literature, the Chenomx NMR Suite 7.5–8.1 software and the metabolite databases Human Metabolome Database (HMDB; http://www.hmdb.ca/)305, Spectral database for Organic Compounds (SDBS; http://riodb01.ibase.aist.go.jp/sdbs/; National Institute of Advanced Industrial Science and Technology, Japan), Yeast Metabolome Database (YMDB; http://www.ymdb.ca/)306. Selected samples were subjected to additional 2D NMR experiments.

4.2.5 Origin-related background information

4.2.5.1 Meteorological data (crops)

The meteorological data used in studies I and IV was collected by the Finnish Meteorological Institute (Helsinki, Finland) and the National Climate Data and
Information Archive of the Environment Canada (Fredericton, New Brunswick, Canada). Weather data for China was unavailable. Data from the weather stations of Kustavi Isokari (latitude 60° N, longitude 21° E, altitude 4 m), Kemi-Tornio Airport (65° N, 24° E, 19 m), Rovaniemi Airport (66° N, 25° E, 195 m) were used for the locations of Uusikaupunki, Kemi and Taapajärvi, respectively. The data from Turku Artukainen (60° N, 22° E, 8 m), Kittilä Pokka (68° N, 25° E, 275 m) and the Jean Lesage International Airport (46° N, 71° W, 74 m) were used for Turku, Kittilä and Québec, respectively.

The meteorological data for the study II for Inkoo, Pernaja, Jokioinen, Hauho and Maaninka was from Espoo Sepänkylä (60° N, 25° E, 31 m), Porvoo Harabacka (60° N, 26° E, 22 m), Jokioinen Observatory (61° N, 24° E, 104 m), Hämeenlinna Lammi Pappila (61° N, 25° E, 125 m) and Maaninka Halola (63° N, 27° E, 90 m), respectively.

The total number of days, effective days (T\text{mean}> +5 °C), hot days (T\text{max}> +25 °C), temperature sum (sum of daily average temperatures), effective temperature sum (degree days; the sum of positive difference between daily average temperatures and +5 °C), global radiation sum, average relative humidity and precipitation sum were calculated for each location. The daily records from the start of the growing season until the harvest date were included. The weather data was used in multivariate modelling after UV-scaling.

4.2.5.2 Melissopalynological analysis (honey)

The botanical roots of the honeys (study III) were surveyed by an expert from the Finnish Beekeepers’ Association using a standard melissopalynological (pollen) test.\textsuperscript{149,307} Circa 400 pollen grains were microscoped, identified and counted for each honey sample. Relative percentages of pollen types were calculated based on the test.

4.2.6 Statistics and multivariate data analysis

The SIMCA-P+ 12.0.1 software (MKS Instruments AB, Umeå/Malmö, Sweden; former Umetrics AB, Umeå, Sweden) was used for the multivariate data analyses. Mean-centring, Pareto-scaling and unit variance (UV) scaling was used in different cases. Principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA) were used to create the multivariate models. In the supervised models, a classification according to subspecies, species, cultivar, botanical origin, or geographical origin was used. The PLS-DA models were validated by performing a permutation test of 20 permutations.

In study I, independent samples $t$-test or Mann–Whitney $U$ test (non-
parametric variables) were used to test statistically significant \((p < 0.05)\) differences within the data. In study IV, Pearson’s correlation coefficients were calculated for selected weather variables and bins.
5 RESULTS AND DISCUSSION

5.1 NMR metabolomics reveals the phenotypic plasticity in sea buckthorn berry composition in respect to genotype × environment interaction

5.1.1 Wild sea buckthorn berries

The berries from Uusikaupunki (60° N; example spectrum in Figure 8) and Kemi (65° N) were easily distinguished by the relatively high levels of ethyl glucoside (2.1–3.3 and 0.7–1.7 g/100 g, fw), whereas berries from Taapajärvi (67° N) had lower levels of the ethyl glucoside (37–180 mg/100 g), but intensive signals of malic (4.9–8.1 g/100 g). The level of glucose, ethyl glucoside, quinic acid and trigonelline were statistically different between the berries from Uusikaupunki and Taapajärvi (p < 0.05), indicating responsiveness to the climate as affected by latitude and phenotypic plasticity among the plants of the same genetic origin. Although the geographical distance between Kemi and Taapajärvi is relatively short, the locations situate at different zones. In the northern temperate watershed area, Kemi’s proximity to the Bothnian Bay affects its climate (middle boreal climate zone). Taapajärvi, above the Arctic Circle, belongs to the northern boreal zone. In comparison, the climate in Uusikaupunki is hemiboreal – generally the most favourable in Finland.

Degree days, symbolising the total energy available for the crop, are defined as the sum of values exceeding the threshold (+5 °C in the Nordic countries). Here, the effective temperature sums of 1210–1340 °Cd were recorded in Uusikaupunki as the highest (Table 2). In the north, the sums were up to 600 °Cd lower. The discrimination of ssp. *rhamnoides* was mainly associated with typically higher temperature, radiation and humidity and lower precipitation in the south, yielding higher levels of ethyl glucoside and glucose, and lower levels of malic, quinic and ascorbic acids. Ascorbic and quinic acids showed positive correlation with the precipitation sum, being representative of the northernmost berries. Seasonal variation in precipitation may also partly explain why the levels of ascorbic acid in the Uusikaupunki berries were 83–104% higher in 2007–2008 compared to 2009–2010.

In China (ssp. *sinensis*), high altitudes had a strong effect on berry composition, correlating positively with ascorbic and malic acids. The profile of sea buckthorn from high altitudes (2000–3000 m) and low latitude (31° N) in Sichuan differed from the other samples of ssp. *sinensis*. The berries from Sichuan contained hardly any sugars, whereas signals of L-quebrachitol and high contents of ascorbic acid dominated the sugar region of the NMR spectra. The berries of Qinghai (36° N, 3115 m) also exhibited higher levels of ascorbic
Fig. 8 The full $^1$H NMR spectrum (sample from Uusikaupunki, Finland, 2009). The area of 5.30–9.30 ppm is zoomed for closer observation. Abbreviations: His, histidine; Phe, phenylalanine; Trg, trigonelline; TSP, trimethylsilyl propionic-$d_4$ acid (standard); Tyr, tyrosine. Reprinted from the original publication$^{303}$ (supplementary), with permission from Elsevier.
Table 2 The sea buckthorn berry samples (*H. rhamnoides* ssp. *rhamnoides*) and their background data.

<table>
<thead>
<tr>
<th>Growth location</th>
<th>Year</th>
<th>Start of growing season</th>
<th>Harvest date</th>
<th>Days in growing season until harvest</th>
<th>Temp. sum (°C)</th>
<th>Degree days (°Cd)</th>
<th>Global radiation sum (× 10^4 kJ/m²)</th>
<th>Average relative humidity (%)</th>
<th>Precipitation sum (mm)</th>
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<td>Sep 29</td>
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a The last eleven days preceding harvest not included in the calculations due to missing data. b Unripe. c Semi-ripe. d Data from substituting weather station of Kaarina Yltöinen (60° 23′ N, 22° 33′ E, 6 m). e The temperature during the four days preceding harvest were recorded below 0 °C. f Additional irrigation was applied due to a dry period in mid-July, 2010.
Results and Discussion

and malic acids. For the berries from Ordos (39° N, 1480 m), the dominating compound was glucose, but the level of malic acid was lowest of all the samples. Visually, the berries from Shanxi (37° N, 1512/2182 m), Heilongjiang (47° N, 210 m) and Hebei (41° N, 818 m) had quite similar profiles. The results were in accordance with the earlier report the berry juice of ssp. *sinensis*.

Methyl glucoside was present in both subspecies at fairly low levels (≤ 0.1 g/100 g). Statistically significant differences between the subspecies were shown by the levels of ascorbic acid (p < 0.01), methyl glucoside (p < 0.01), L-quebrachitol (p < 0.01) and phenylalanine (p < 0.05). The PCA, explaining 63% of the total variance by the first two principal components, showed excellent goodness-of-fit (R²cum = 0.93) and sound predictive ability (Q²cum = 0.78).

Full discrimination between the subspecies was achieved with the first (X: 26.5%, Y: 56.2%) and third (X: 13.7%, Y: 16.7%) component in PLS-DA, owing to the strong variable influence of ethyl glucoside and malic acid for ssp. *rhamnoides* (Finland) and ssp. *sinensis* (China), respectively. For the Chinese berries, fructose was also a positive correlation. Fructose had a negative weight on both the first and second PLS components.

Compared to earlier reports based on chromatographic analysis of the sea buckthorn berries, both advantages and disadvantages can be stated for NMR metabolomics. Whereas NMR allows a wider range of metabolites to be examined in a single analysis without derivatisation, fractionation and need for standard compounds, the analysis of inositols, fructose and citric acid is challenged by overlapping signals. Still, even though these resonances would visually seem obstructed, the multivariate power is generally able to extract the latent information within the NMR data.

5.1.2 Cultivars 'Terhi' and 'Tytti'

The two cultivars, although possessing very similar traits because of their genetic background, were discriminated by relatively higher levels of quinic acid in the berries of 'Terhi' and ethyl glucoside in 'Tytti'. Malic acid was also present at higher levels in 'Tytti'. Both cultivars were generally low in fructose, compared to the wild berries of the same subspecies or ssp. *sinensis* in study I. Overall, Finnish berries of ssp. *rhamnoides* are low in total sugar compared to other subspecies of Russian or Chinese origin.

The metabolic profiles of the berries of cv. 'Terhi' and 'Tytti' varied greatly between northern (Kittilä, 68° N) and southern Finland (Turku, 60° N) and Canada (Québec, 46° N). The PCA loadings suggested positive correlations between Kittilä and quinic acid (bin 2.02) and Québec and ethyl glucoside (1.23, 4.48). Turku on the other hand correlated negatively with malic acid (2.86). Berries from the high latitude had relatively higher levels of quinic acid,
glucose, L-quebrachitol and ascorbic acid. Kittilä samples contained much lower levels of ethyl glucoside compared to berries from Turku or Québec. Relatively higher levels of free essential amino acids (phenylalanine, tyrosine, tryptophan and valine) were also detected in the Kittilä berries. As in study I, phenylalanine correlated with high latitude, underpinning its role as a precursor for phenolic compounds in the phenylpropanoid pathway. Berries from the Turku correlated positively with ethyl glucoside and negatively with malic acid. Berries from Québec correlated positively with malic acid and negatively with quinic acid and ethyl glucoside. Whereas the relative air humidity during the growth season has been shown to correlate positively with ethyl glucose, methyl-myoinositol, malic acid and total acid in wild sea buckthorn (study I) and berries of ssp. mongolica, with 'Terhi' and 'Tytti' such trend was not seen.

Ethyl glucoside, characteristic to ssp. rhamnoides, did not accumulate during ripening in the north, unlike in the south. The southern growth conditions, especially degree days and global radiation, correlated positively (r = 0.63 and r = 0.59, respectively) with ethyl glucoside. The average natural daily photoperiod during the growth season (until ripe) was 10 h (ranging 0–18 h) in Turku and 6 h (0–20 h) in Kittilä. While the highest daily sunlight period was recorded in Kittilä, the daily average was lower than in the south. Parallel to Taapajärvi and Uusikaupunki in study I, Kittilä and Turku belong to the northern boreal and hemiboreal zone, respectively. Ethyl glucoside was shown to accumulate six-fold at the late stage of maturation in the south. The overripe berries were also characterised by lower levels of glucose, malic acid and quinic acid, and higher levels of phenylalanine, tyrosine, choline and uridine. The already low levels of ethyl glucose in Kittilä did not increase in overripe berries, indicating that the northern conditions do not favour the formation of the metabolite. The high content of ethyl glucoside in the overripe berries suggests that the compound may act as either a storage compound or as a protective compound against heat/light stress in the south and/or ethanol-induced toxicity.

5.2 The influence of genotype, growth conditions and developmental stage on Brassica oilseeds

5.2.1 Ripened seeds

In addition to the major lipids present in the cyclohexane extracts, choline (in CDCl$_3$) with sucrose and sinapic acid esters (in CD$_3$OD, Table 3), the minor seed components generally lost during oil processing and refining, were detected. A higher content of polyunsaturated fatty acids and sucrose were observed in turnip rape, while the overall oil content and sinapine levels were...
Table 3 Chemical shift assignments of the $^1$H NMR signals from the methanol extracts with HSQC correlations.

<table>
<thead>
<tr>
<th>Multiplicity$^a$</th>
<th>Chemical shift (ppm)</th>
<th>$J$ (Hz)</th>
<th>HSQC correlation (ppm)</th>
<th>Functional group</th>
<th>Attribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>s</td>
<td>0.72</td>
<td></td>
<td></td>
<td>CH$_{3}$-18</td>
<td>sterol</td>
</tr>
<tr>
<td>s</td>
<td>0.73</td>
<td></td>
<td></td>
<td>CH$_{3}$-18</td>
<td>sterol</td>
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<tr>
<td>m</td>
<td>0.84</td>
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<tr>
<td>t</td>
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<td>7.2</td>
<td>13.2</td>
<td>CH$_{3}$</td>
<td>saturated fatty acids, oleic acid</td>
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<tr>
<td>t</td>
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<td></td>
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<td>linoleic acid</td>
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<tr>
<td>t</td>
<td>0.97</td>
<td>7.5</td>
<td></td>
<td>CH$_{3}$</td>
<td>$\alpha$-linolenic acid</td>
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<tr>
<td>d</td>
<td>1.09</td>
<td>6.9</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>d</td>
<td>1.15</td>
<td>6.2</td>
<td></td>
<td>(CH$_{2}$)$_n$</td>
<td>acyl</td>
</tr>
<tr>
<td>s</td>
<td>1.22</td>
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<td></td>
<td>OCO-CH$_2$CH$_3$</td>
<td>acyl</td>
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<tr>
<td>m</td>
<td>2.04</td>
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<td>CH$_2$-CH=CH</td>
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<tr>
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<td>OCO-CH$_2$:</td>
<td>acyl</td>
</tr>
<tr>
<td>t</td>
<td>2.77</td>
<td>6.3</td>
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<td>linoleic</td>
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<td>5.8</td>
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<td>$=CH$-CH$_2$:CH=</td>
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<tr>
<td>s</td>
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<tr>
<td>dd/t</td>
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<tr>
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<td>sucrose ($\alpha$-Glc)</td>
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<tr>
<td>d</td>
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<td>sucrose ($\beta$-Fru$^c$)</td>
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<tr>
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<td>12.2</td>
<td>62.7</td>
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<td>sucrose ($\beta$-Fru)</td>
</tr>
<tr>
<td>m</td>
<td>3.71</td>
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<td>73.0</td>
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<td></td>
<td>CH-5</td>
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<td>55.5</td>
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</tr>
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<td>74.1</td>
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</tr>
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<td>77.9</td>
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<td>sucrose ($\beta$-Fru)</td>
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<td>dd</td>
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<td>glycerol (sn-1/3)</td>
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<td>4.36</td>
<td>3.8; 12.0</td>
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<tr>
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<td>CH-OCOR</td>
<td>glycerol (sn-2)</td>
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<td>3.9</td>
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<td>raffinose/stachyose ($\alpha$-Glc)</td>
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<td>105.7</td>
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<td>6.93</td>
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<td></td>
<td>CH-(\alpha)</td>
<td>sinapine</td>
</tr>
<tr>
<td>d</td>
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<td>15.9</td>
<td></td>
<td>CH-(\alpha)</td>
<td>sinapic acid ester</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>s</td>
<td>8.09</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Multiplicity: s, singlet; d, doublet; t, triplet; dd, doublet of doublets; m, multiplet; br, broad signal. $^b$ $\alpha$-Glc, $O$-$\alpha$-$D$-glucopyranosyl. $^c$ $\beta$-Fru, $\beta$-$D$-fructofuranoside.
higher in oilseed rape. However, absolute discrimination among the oilseed species was not established here. The effect of genotype on the oilseed metabolome at cultivar-level was not included within this study as the cultivar traits were negligible compared to the influence of species and, to some extent, growth place. In PCA applied to the cyclohexane extracts, the weight of the allylic methylene bin of 2.05 indicated a higher content of PUFAs firstly in *B. rapa* and especially in the northernmost location, Maaninka. Both observations were consistent with prior knowledge as low temperature is known to increase the level of ALA. High content of ALA may be desirable for dietary reasons, yet, as it is easily oxidised, the quality and shelf life of the oil can be decreased and the possible health benefits adversed.

In PCA applied to the CD$_3$OD extract data, the loadings of the third component revealed the key metabolites discriminating the species as sinapine for *B. napus* and polyunsaturated fatty acids and sucrose for *B. rapa*. The average daily temperatures of 16–17 °C were recorded in all location, while the highest and lowest temperature sums were in Pernaja (*B. napus*, 2130 °C) and Maaninka (*B. rapa*, 1310 °C), respectively. The OPLS-DA applied to the CD$_3$OD extracts revealed a positive correlation among the samples from Pernaja and Maaninka, owing to sucrose and cholinyl resonances. The growth sites on zone II correlated each other, likely owing to their close proximity and similar weather conditions. In that respect, since oilseed rape generally matures slower than turnip rape, the relatively higher temperature and radiation conditions in Pernaja may have promoted the seed maturation as to correspond the compositional characteristics of turnip rape grown in Maaninka.

### 5.2.2 Developing seeds

The NMR profiles of the two oilseed rape and two turnip rape genotypes revealed the metabolic differences in optimal and cold conditions at different stages of early seed development. During the first weeks after flowering, the degree of unsaturation (δ 0.97 and 1.30 ppm, *Figure 9*) was shown to increase more in relation to the overall lipid content (δ 0.88 and 1.25 ppm). The lipid composition changed more between 2 and 3 WAF than between 3 and 4 WAF, indicating that the desaturation is activated at this stage after the primary fatty acid biosynthesis. Concurrently, a decrease in the terminal methyl resonance at δ 0.83 ppm occurred. Although the sampling of the siliques from the greenhouse (15–20 °C, 16–19 h), growth room (22 °C, 16 h) and low-temperature (15 °C, 16 h) test was uneven, the PCA modelling showed several trends along the first component ($R^2_X[1] = 0.88$, $Q^2[1] = 0.86$). The same trend was seen as in *Figure 9*, as the 2 WAF and 3–4 WAF samples were split on positive and negative sides of the component, respectively. The respective loadings indicated a relatively lower level of acyl (–CH$_2$–) resonances
corresponding those of saturated and oleic acids (δ 1.20 and 1.24 ppm) in 2 WAF and relatively higher levels of acyl resonances of polyunsaturated fatty acids (δ 1.28 and 1.32 ppm) in 3 and 4 WAF. The low-temperature treatment mimicking the effect of growth conditions in northern latitudes delayed the development by a week and correlated with the higher level of unsaturation. The results were consistent with the previous findings of increasing expression levels of acyltransferase, elongase and desaturase genes DGAT1–3, FAE1 and FAD3 from the 3 or 4 WAF time point on.21

![Diagram of lipid profile](image)

**Fig. 9** An example of the effect of seed development stage on lipid profile. The aliphatic range in this figure shows some of the differences between the seeds picked 2 weeks after flowering (black spectra) and the seeds picked 3 or 4 weeks after flowering (grey spectra) affect. As the seed develops, the content of polyunsaturated fatty acids increases, while the 0.83 ppm resonances decrease. Reprinted from the original publication304 (supplementary), with permission from Elsevier.
5.3 **Botanical origin of honey is elucidated by NMR fingerprints and multivariate modelling**

According to the melissopalynological data (Table 4), many of the samples contained pollen from Apiaceae (e.g. angelicas), Brassicaceae (oilseed rape, turnip rape), *Rubus* spp. (mainly raspberry), *Salix* spp. (willows) and *Trifolium* spp. (white/alsike and red clover), of which the pollen of Apiaceae and Brassicaceae is generally over-represented. These are generally the most prevalent pollen types found in Finnish honeys. In the pollen analysis, honeydew honeys were characterised by the presence of fungal spores, hyphae and microscopic algae at a ratio of honeydew elements-to-pollen grains exceeding three (3). With NMR, melezitose and trehalose, with fumaric, lactic, malic, and succinic acids were assigned markers of honeydew. Asparagine and aspartic acid were also present in honeydew honey at detectable levels. The marker present in the organic extract, diacylglycerol ether, complimented the results. In PCA, the data polarised into honeydew and floral honeys as the honeydew-specific saccharide melezitose, and glucose and fructose for floral honeys had the strongest influence on the distribution. The first two principal components explained 83% of the total variation in the PCA model ($R^2_X$ (cum) = 0.99 and $Q^2$ (cum) = 0.96).

While the dandelions (*Taraxacum* spp.) are commonly available for foraging and rich in nectar, only few grains of pollen can be found in honeys. Here, 0–2.4% of the pollen in the dandelion honeys was from *Taraxacum*, highlighting the ambiguity of pollen-based identification. In fact, most of the honeys contained regulatory-wise insufficient levels (<45%) of pollen from the signified botanical origin. The second principal component in PCA could be seen as an approximate indicator of fructose-to-glucose (F/G) ratio and thus the possible origin of nectar. Low F/G ratio indicates readiness to crystallisation. Honeys with high F/G ratio, on the other hand, stay liquid. Based on the PCA, dandelion honey had the highest relative content of glucose, consistent with prior knowledge. Novel markers in dandelion honeys were detectable in the aliphatic region of the spectra and identified as 2-hydroxy-3-methylbutyric, 2-hydroxy-3-methylpentanoic, 3-methyl-2-oxopentanoic and 4-methyl-2-oxopentanoic acids (Figure 10, Figure 11). These metabolites were present at low levels and varying proportions. Although the structures of these compounds suggest that they could be products of microbial fermentation, they still can be regarded as specific for dandelion honey. Unlike dandelion honey, clover honey was distinguished by the relatively high and low levels of fructose and glucose, respectively. Buckwheat pollen can be regarded as under-represented pollen types, consistent with previous knowledge. The levels of
Table 4 Botanical and geographical origin of the honey samples.

<table>
<thead>
<tr>
<th>No.</th>
<th>Botanical origin</th>
<th>Other pollen types present (≥ 3%)</th>
<th>Geographical origin</th>
<th>Year of harvest</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Buckwheat (<em>Fagopyrum esculentum</em>, 1.7%)</td>
<td><strong>Brassicaceae</strong>, 71%; <strong>Trifolium repens/hybridum</strong>, 10%; <strong>Salix spp.</strong>, 6.5%; <strong>Trifolium pratense</strong>, 4.1%</td>
<td>60° N, 23° E</td>
<td>2012</td>
</tr>
<tr>
<td>2</td>
<td>Buckwheat (<em>F. esculentum</em>, 1.7%)</td>
<td><strong>Brassicaceae</strong>, 36%; <strong>Salix spp.</strong>, 32%; <strong>Apiaceae</strong>, 5.1%; <strong>T. repens/hybridum</strong>, 4.6%; <strong>Vicia faba</strong>, 3.1%</td>
<td>62° N, 25° E</td>
<td>2013</td>
</tr>
<tr>
<td>3</td>
<td>Dandelion (<em>Taraxacum spp.</em>, 0.2%)</td>
<td><strong>Brassicaceae</strong>, 52%; <strong>Salix spp.</strong>, 31%; <strong>Rubus spp.</strong>, 5.3%; <strong>Apiaceae</strong>, 4.3%</td>
<td>60° N, 21° E</td>
<td>2012</td>
</tr>
<tr>
<td>4</td>
<td>Dandelion (<em>Taraxacum spp.</em>, 2.4%)</td>
<td><strong>Brassicaceae</strong>, 44%; <strong>Rubus spp.</strong>, 24%; <strong>Salix spp.</strong>, 15%; <strong>Sorbus spp.</strong>, 3.6%</td>
<td>64° N, 24° E</td>
<td>2013</td>
</tr>
<tr>
<td>5</td>
<td>Dandelion (<em>Taraxacum spp.</em>, 0.7%)</td>
<td><strong>Salix spp.</strong>, 73%; <strong>Rubus spp.</strong>, 9.4%; <strong>Sorbus spp.</strong>, 9.4%; <strong>Malus/Pyrus spp.</strong>, 5.2%</td>
<td>62° N, 30° E</td>
<td>2013</td>
</tr>
<tr>
<td>7</td>
<td>Honeydew*</td>
<td><strong>Rubus spp.</strong>, 34%; <strong>Impatiens spp.</strong>, 33%; <strong>Salix spp.</strong>, 15%; <strong>T. repens/hybridum</strong>, 8.9%; <strong>Apiaceae</strong>, 5.0%; <strong>Parthenocissus</strong>, 3.9%</td>
<td>62° N, 25° E</td>
<td>2013</td>
</tr>
<tr>
<td>8</td>
<td>Clover (<em>T. repens/hybridum</em>, 63%)</td>
<td><strong>Salix spp.</strong>, 8.5%; <strong>Rubus spp.</strong>, 6.5%; <strong>Brassicaceae</strong>, 6.1%; <strong>Filipendula spp.</strong>, 4.6%</td>
<td>62° N, 29° E</td>
<td>2012</td>
</tr>
<tr>
<td>9</td>
<td>Linden (<em>Tilia spp.</em>, 29%)</td>
<td><strong>Brassicaceae</strong>, 19%; <strong>Filipendula spp.</strong>, 15%; <strong>Phacelia spp.</strong>, 11%; <strong>T. repens/hybridum</strong>, 8.8%; <strong>Rubus spp.</strong>, 4.9%</td>
<td>60° N, 24° E</td>
<td>2012</td>
</tr>
<tr>
<td>10</td>
<td>Heather (<em>Calluna vulgaris</em>, 14%)</td>
<td><strong>Brassicaceae</strong>, 51%; <strong>T. repens/hybridum</strong>, 18%; <strong>Apiaceae</strong>, 10%; <strong>Fabaceae</strong>, 3.4%</td>
<td>60° N, 25° E</td>
<td>2013</td>
</tr>
<tr>
<td>11</td>
<td>Heather (<em>C. vulgaris</em>, 18%)</td>
<td><strong>Brassicaceae</strong>, 54%; <strong>T. repens/hybridum</strong>, 17%; <strong>Apiaceae</strong>, 5.5%; <strong>Filipendula spp.</strong>, 4.3%</td>
<td>61° N, 21° E</td>
<td>2012</td>
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<td><strong>Brassicaceae</strong>, 37%; <strong>T. repens/hybridum</strong>, 30%; <strong>Rubus spp.</strong>, 3.9%; <strong>Salix spp.</strong>, 3.6%</td>
<td>63° N, 23° E</td>
<td>2013</td>
</tr>
<tr>
<td>13</td>
<td>Honeydew*</td>
<td><strong>Salix spp.</strong>, 38%; <strong>T. repens/hybridum</strong>, 26%; <strong>Brassicaceae</strong>, 16%; <strong>Apiaceae</strong>, 8.3%; <strong>C. vulgaris</strong>, 4.9%; <strong>Filipendula spp.</strong>, 4.2%</td>
<td>64° N, 24° E</td>
<td>2013</td>
</tr>
<tr>
<td>14</td>
<td>Himalayan balsam (*Impatiens glandulifera, 25%)</td>
<td><strong>Brassicaceae</strong>, 40%; <strong>Rubus spp.</strong>, 11%; <strong>Salix spp.</strong>, 4.9%; <strong>Filipendula spp.</strong>, 3.9%; <strong>T. repens/hybridum</strong>, 3.9%</td>
<td>64° N, 24° E</td>
<td>2013</td>
</tr>
<tr>
<td>15</td>
<td>Multifloral</td>
<td><strong>Brassicaceae</strong>, 44%; <strong>Rubus spp.</strong>, 37%; <strong>Salix spp.</strong>, 4.6%; <strong>T. repens/hybridum</strong>, 5.6%</td>
<td>60° N, 24° E</td>
<td>2013</td>
</tr>
<tr>
<td>16</td>
<td>Lingonberry (<em>Vaccinium spp.</em>, 16%)</td>
<td><em>Rubus spp.</em>, 40%; <strong>Myosotis</strong>, 22%; <strong>Salix spp.</strong>, 11%</td>
<td>63° N, 28° E</td>
<td>2013</td>
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<td>17</td>
<td>Multifloral</td>
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<td>60° N, 24° E</td>
<td>2013</td>
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<td>18</td>
<td>Dandelion (<em>Taraxacum spp.</em>, 0.0%)</td>
<td><strong>Brassicaceae</strong>, 35%; <strong>Apiaceae</strong>, 33%; <strong>Salix spp.</strong>, 21%; <strong>Rubus spp.</strong>, 5.0%; <strong>T. repens/hybridum</strong>, 4.2%</td>
<td>not specified</td>
<td>2013</td>
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<tr>
<td>19</td>
<td>Heather (<em>C. vulgaris</em>, 3.3%)</td>
<td><strong>Brassicaceae</strong>, 32%; <strong>Melilotus</strong>, 24%; <strong>T. repens/hybridum</strong>, 8.8%; <strong>Apiaceae</strong>, 7.0%; <strong>Fabaceae</strong>, 9.3%; <strong>Filipendula spp.</strong>, 5.6%; <strong>Rubus spp.</strong>, 4.9%; <strong>Salix spp.</strong>, 3.7%</td>
<td>not specified</td>
<td>2013</td>
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<tr>
<td>20</td>
<td>Buckwheat (<em>F. esculentum</em>, 7.7%)</td>
<td><strong>Brassicaceae</strong>, 62%; <strong>Salix spp.</strong>, 15%; <strong>Apiaceae</strong>, 6.3%; <strong>Fabaceae</strong>, 3.1%</td>
<td>not specified</td>
<td>2013</td>
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</tbody>
</table>

*a* Under-represented pollen type.\(^{152,312}\) *b* Over-represented pollen type.\(^{152,312}\) *c* Honeydew elements.\(^{149}\) *d* Non-melliferous plant.\(^{131}\)
Results and Discussion

Fig. 10 Low-intensity, high-field fingerprints for the featured heather, buckwheat, dandelion and linden honeys (overlapping spectra of samples 1–5, 9–12 and 18–20).

Fig. 11 Compounds suggested as novel markers of *Taraxacum* spp. honey. a) 2-hydroxy-3-methylbutyric acid, b) 2-hydroxy-3-methylpentanoic acid, c) 3-methyl-2-oxopentanoic acid and d) 4-methyl-2-oxopentanoic acid.

Isoleucine, leucine and valine were highest in buckwheat honeys (Figure 10). Also, threonine, tyrosine and 4-hydroxybenzoic acid with some unassigned signals at δ 5.04, 6.67, 6.70 and 6.80 ppm were characteristic to buckwheat. Heather honeys were marked by 3-phenyllactic and phenylacetic acid, together with relatively higher amounts of acetic, benzoic, formic, phenyllactic and pyruvic acid and proline. In addition, dehydrovomifoliol present in the
Results and Discussion

organic extract was specific to heather. The pair-wise discriminations (OPLS-DA; $R^2 > 0.9$, $Q^2 > 0.9$) of buckwheat, dandelion and heather honeys emphasised the weight of abovementioned botanical markers and the relative content of glucose and fructose. Linden honey was authenticated by a known marker, \(1-O-\beta\)-gentiobiosyl \((6-O-(\beta-D\text{-glucopyranosyl})-\beta-D\text{-glucopyranosyl})\) ester of 4-(1-hydroxy-1-methylethyl)cyclohexa-1,3-diene-1-carboxylic acid (also as respective aglycone in CDCl$_3$), and a relatively high proportion of gentiobiose.$^{313}$ Lingonberry honey contained relatively high content of methyl syringate$^{311}$ (in CDCl$_3$) and was also characterised by unassigned peaks at the aromatic region, suggesting that these signals originate from a compound representative of *Vaccinium* spp. As for clover and Himalayan balsam honey, there were not any specific aqueous markers to be declared for lingonberry honey.

Incorrect classification of one heather and one Himalayan balsam honey was revealed with NMR, and they were subsequently denoted as honeydew (sample 13) and multiforal honey (sample 15), respectively. The botanical origin of the samples 18–20 (of commercial and therefore of unspecified geographical origin) were confirmed to match the labelling. However, the levels of the key botanical markers were lower than in the other respective honeys, possibly due to handling and storage conditions or careless harvest.

As the first steps in NMR metabolomics-based characterisation of Finnish varietal honeys, this study successfully found clear discriminative basis to which future unknown samples could be referenced. However, to upgrade predictive power of the models, the dataset should be complemented with other honey varieties and more samples to construct a comprehensive national honey library. Also, as pure varietal honeys rarely exist naturally, experiments involving caged beehives on unifloral patches would be required in order to accommodate the requirements. Until then, the method is not fully comprehensive for routine quality control but still applicable in the qualitative characterisation of buckwheat, dandelion, heather, honeydew and linden honeys.
The experimental part of the thesis concentrated on the NMR metabolomics of sea buckthorn berries and *Brassica* oilseeds as crops of varying genotypes grown in varying locations and environmental conditions, and honey, as an example of food deriving its characteristics from the boreal nature but often being subject to mislabelling and fraudulence.

Genetic and climatic factors significantly affect the composition and quality of sea buckthorn berries. Berries of same genetic origin may exhibit drastically different phenotypes in different climates and growth conditions as affected by latitude and altitude. Glucose, ethyl glucose, malic acid, quinic acid and ascorbic acid are the key metabolites in sea buckthorn berries influenced by the growth place. The northern growth conditions produce more vitamin C and precursors for other secondary metabolites for protective compounds against abiotic stress factors related to the high latitude. Significant metabolic differences in genetically identical berries were observed between latitudes 60° and 67–68° north in Finland. High altitudes (> 2000 m) correlated with greater levels of malic and ascorbic acids in *ssp. sinensis*. The NMR metabolomics approach applied here is effective for identification of metabolites, geographical origin and subspecies of sea buckthorn berries.

In oilseed rape and turnip rape, the genotype at species-level and the growth place were the main determinants for the seed composition. Differences in the major lipids and the minor metabolites between the two species were found. A higher content of polyunsaturated fatty acids and sucrose were observed in turnip rape. The overall oil content and sinapine levels were higher in oilseed rape. The effect of cultivar type on the oilseed metabolome was considered negligible compared to the effect of the growth place and the associated conditions. No clear trends among cultivars were shown, however, the combined cultivar × environment interaction cannot be ruled out.

The NMR–multivariate approach allowed the full characterisation of Finnish buckwheat, dandelion and heather honeys, whose true botanical origin is grossly understated in the pollen analysis. Novel markers were suggested for characterisation of the dandelion honey. The metabolic fingerprints and the fructose-to-glucose ratio will also promote the identification of other honey types, such as clover, honeydew and linden honey. The methodological power of NMR metabolomics in finding and identifying botanical markers originating from the floral/nectar source rivals the existing set of established tools in honey analysis and encourages further developments aiming towards a national honey library.

The studies included in this thesis demonstrated the applicability of NMR-based analysis in determining the some of the key metabolic compounds of
different foods and raw ingredients in respect to the food origin (especially the Nordic dimension). This research provides novel approaches and tools for food quality control and plant breeding, while supporting the selection of raw ingredients aimed at optimal sensory and chemical properties for the food and nutraceutical industry. The knowledge on crop quality and differences between species, cultivars, growth sites and maturity in order to find the optimum conditions for producing profitable quality crops may rise to even greater importance in the near future, considering climatic changes. Assisting in agricultural and breeding processes, metabolomics can help in the selection of target genotypes and cultivation sites for enrichment of bioactive components or sensory properties. Also, consumer awareness towards food origin and authenticity and the need for efficient tools to verify them have increased. The methodologies used here could potentially be extended to be used in detecting food frauds/adulterations, quality defects and genetically modified crops. In that respect, it is essential to characterise the key metabolites that unmistakably define and differentiate foods, as also shown here. However, the models and spectral libraries to which future samples are compared need to be complemented with and representative of a wide range of samples and their variations in order to exhibit their full performance in internationally valid quality control. NMR metabolomics is an essential tool in understanding metabolic profiles of foods that are effected by different environmental factors and, in due course, reflecting to consumer acceptance and trust in the global food markets.
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REFERENCES


37. Mannina, L.; Sobolev, A.P.; Viel, S. Liquid state 1H high field NMR in food analysis.
References


64. Marseglia, A.; Acquotti, D.; Consonni, R.; Cagliani, L.R.; Palla, G.; Caligiani, A. HR MAS 1H NMR and chemometrics as useful tool to assess the geographical origin of cocoa beans – Comparison with HR 1H NMR. *Food Res. Int.* 2016, 85, 273–281.


References


95. Korekar, G.; Dolkar, P.; Singh, H.; Srivastava, R.B.; Stobdan, T. Variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of Seabuckthorn (Hippophae
References


113. Yang, B.; Karlsson, R.M.; Oksman, P.H.; Kallio, H.P. Phytosterols in sea buckthorn (Hippophae rhamnoides L.) berries: Identification and effects of different...
References


156. Svečnjak, L.; Bubalo, D.; Baranović, G.; Novosel, H. Optimization of FTIR-ATR spectroscopy for botanical authentication of


177. Mallamace, D.; Corsaro, C.; Salvo, A.; Cicero, N.; Macaluso, A.; Giangrosso, G.; Ferrantelli, V.; Dugo, G. A multivariate statistical analysis coming from the NMR metabolic profile of cherry tomatoes (the


197. Brennan, L. NMR-based metabolomics: From sample preparation to applications in


on three different species tibetan medicine sea buckthorn by \textsuperscript{1}H-NMR-based metabonomics. \textit{Zhongguo Zhong Yao Za Zhi} 2014, 39, 4234–4239.


258. Sacco, A.; Brescia, M.; Liuzzi, V.; Reniero, F.; Guillou, G.; Ghelli, S.; Meer, P. Characterization of italian olive oils...
References


271. Rochfort, S.; Ezernieks, V.; Bastian, S.E.P.; Downey, M.O. Sensory attributes of wine influenced by variety and berry shading discriminated by NMR metabolomics. Food Chem. 2010, 121, 1296–1304.


276. Petrakis, E.A.; Cagliani, L.R.; Polissiou, M.G.; Consomni, R. Evaluation of saffron (Crocus sativus L.) adulteration with plant...


References


DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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.
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29. MARI HAKALA (2002) Factors affecting the internal quality of strawberry (Fragaria x ananassa Duch.) fruit.
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