DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

Metabolomics-based approach to study the impact of food processing on plant-based protein-rich foods

JASMIN RAITA

Food Sciences
Department of Life Technologies

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

Metabolomics-based approach to study the impact of food processing on plant-based protein-rich foods

JASMIN RAITA

Food Sciences
Department of Life Technologies

TURKU, FINLAND – 2026

University of Turku
Department of Life Technologies, Faculty of Technology
Food Sciences
Doctoral Programme in Technology

Supervised by
Professor Kati Hanhineva, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

Docent Ville Koistinen, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

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

Reviewed by
Assistant Professor Stephan Van Vliet, Ph.D.
Department of Nutrition, Dietetics, and Food Sciences
Utah State University
Logan, Utah, United States

Associate Professor Ndegwa H. Maina, Ph.D.
Department of Food and Nutrition
University of Helsinki
Helsinki, Finland

Opponent
Professor Hanne Christine Bertram, Ph.D.
Department of Food Sciences
Aarhus University
Aarhus, Denmark

Research director
Professor Baoru Yang, Ph.D.
Department of Life Technologies
University of Turku
Turku, Finland

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

ISBN 978-952-02-0681-9 (print)
ISBN 978-952-02-0682-6 (pdf)
ISSN 2323-9395 (print)
ISSN 2323-9409 (pdf)
Painosalama Oy – Turku, Finland 2026

Jos ei niinku jaksa niin koittakaa vaan jaksaa
niilo22

TABLE OF CONTENTS

ABSTRACT

Plant-based diets (PBD) that consist of whole grains, fruits, berries, vegetables, nuts, seeds, and legumes have been shown to yield positive health outcomes. In addition to the beneficial impact, PBD can be more sustainable for the planet. The production of animal-based proteins is the main source of greenhouse gas emissions in agriculture, and the consumption of highly processed meat has been associated with negative health outcomes. Therefore, plant-based protein-rich (PBPR) options are needed. Plant-based foods are often processed in various ways to improve their nutritional content and sensory properties. Processing techniques like cutting and peeling can be gentler on the raw material, while extraction of components can be more intense. During processing some biochemical changes can often occur, such as changes in the structures and amounts of existing compounds and formation of new compounds. In addition, unwanted compounds can be removed and beneficial compounds lost from the raw material.

The aim of this thesis was to examine the composition of small molecules in plant-based protein-rich foods and the impact of various food technological processing techniques on them. A metabolomics approach using liquid chromatography with mass spectrometry (LC–MS) was used with reversed-phase (RP) and hydrophilic interaction chromatography (HILIC) to cover a wide range of metabolites. The first study focused on commercially available PBPR foods where products made from different raw materials with varying processing techniques were analysed. In addition, existing food processing classification systems were used to assess whether they agree with the study findings regarding PBPR foods. In the second study, four different fermentation techniques were used to produce lupin-based beverages and changes in their biochemical compositions were analysed with the same metabolomics approach.

The findings from both of these studies show that food processing can have major effects on the biochemical compounds present in plant-based foods. Fermentation stood out as a promising technique to possibly improve the bioavailability of beneficial compounds in soy-based foods in the first study, whereas these same compounds were almost absent in products made with purified protein concentrates or isolates. When the existing food processing classifications were used with these products, most of the foods were categorised as so-called ultra-processed foods, and some conflicting results were observed. In lupin-based beverages, the different fermentation techniques increased the abundances of lactic acid derivatives and decreased the abundances of vitamin B compounds. By selecting the most suitable fermentation technique depending

on the desired results, lupins could be used to produce protein-rich alternatives to animal-based milk in the future.

The amount of plant-based foods in the diet, especially legumes, vegetables and fruits, should be increased for both environmental and health reasons. While the consumption of plant-based foods is generally beneficial, the impact of foods consisting of refined plant-based materials on human health is not fully understood yet. Plant-based foods often require processing and its impact on the comprehensive biochemical composition of these foods needs to be studied in greater detail. When assessing the healthiness of PBPR foods it would be important to consider the content and profile of nutrients and other potentially health-beneficial biochemicals over their possible classification categories, as plant-based foods with beneficial compounds can often be classified as “ultra-processed”. This thesis provides valuable insight into these aspects, and in particular demonstrates the possibilities of food fermentation to develop PBPR foods.

SUOMENKIELINEN ABSTRAKTI

Kasvipohjaiset ruokavaliot, jotka koostuvat täysjyvistä, marjoista, hedelmistä, vihanneksista, pähkinöistä, siemenistä ja pavuista, ovat osoittaneet positiivisia terveysvaikutuksia. Näiden hyödyllisten vaikutusten lisäksi kasvipohjaisten ruokavalioiden ympäristövaikutukset voivat olla planeetalle kestävämpiä. Eläinperäisten proteiinien tuotanto on yksi maatalouden kasvihuonepäästöjen suurimmista lähteistä, ja prosessoidut lihavalmisteet on yhdistetty kielteisiin terveysvaikuksiin. Lihatuotantoon liittyvien haasteiden vuoksi tulevaisuudessa tarvitaan proteiinipitoisia kasvipohjaisia vaihtoehtoja. Kasvipohjaiset tuotteet ovat usein prosessoituja niiden ravitsemuksellisten ja aistittavien ominaisuuksien parantamiseksi. Prosessointimenetelmät kuten pilkkominen ja kuoriminen ovat hellävaraisempia menetelmiä kuin tiettyjen komponenttien erottaminen kasveista. Prosessoinnin aikana tapahtuu usein muutoksia tuotteen biokemiallisessa koostumuksessa, kuten uusien yhdisteiden muodostumista ja tuotteessa jo ennestään olleiden yhdisteiden rakenteiden kemiallisia muutoksia. Lisäksi voidaan menettää sekä ei-toivottuja että hyödyllisiä yhdisteitä.

Tämän väitöskirjan tarkoituksena oli tutkia kasvipohjaisten proteiinirikkaiden tuotteiden pienmolekyylisten yhdisteiden koostumusta ja erilaisten prosessointimenetelmien vaikutusta siihen. Työssä käytetty metabolomiikkatekniikka perustui nestekromatografia massaspektrometriaan käyttäen käänteisfaasi- ja hydrofiilisen vuorovaikutuksen kromatografista erottelua, jolloin metaboliitteja saatiin analysoitua mahdollisimman laajasti. Ensimmäisessä tutkimuksessa keskityttiin eri tavoin valmistettujen kaupallisten proteiinipitoisten kasvipohjaisten tuotteiden analysointiin. Lisäksi tutkimuksessa selvitettiin olemassa olevien ruoan prosessoinnin luokittelujärjestelmien soveltuvuutta kasvipohjaisiin tuotteisiin ja tutkimuksessa saatuihin tuloksiin. Toisessa tutkimuksessa käytettiin samaa metabolomiikkatekniikkaa neljän eri fermentointitavan vaikutuksen selvittämiseksi lupiinista valmistettujen juomien biokemialliseen koostumukseen.

Molempien tutkimusten tulokset osoittavat, että ruoan prosessoinnilla voi olla suuri vaikutus kasvipohjaisten tuotteiden biokemialliseen koostumukseen. Ensimmäisessä tutkimuksessa fermentointi erottui lupaavana tekniikkana, joka voi mahdollisesti parantaa hyödyllisten yhdisteiden saatavuutta soijapohjaisissa tuotteissa, kun taas nämä samat yhdisteet puuttuivat lähes kokonaan tuotteista, jotka oli valmistettu proteiinikonsentraateista tai -isolaateista. Kun ruoan prosessoinnin luokittelujärjestelmiä käytettiin näille samoille tuotteille, niin useimmat niistä luokiteltiin niin kutsutuiksi ultraprosessoiduiksi tuotteiksi ja

verrattaessa biokemialliseen koostumukseen havaittiin ristiriitaisia tuloksia. Lupiinipohjaisiin juomiin eri fermentointitekniikat vaikuttivat esimerkiksi lisäämällä maitohappojohdannaisten signaalien tasoja ja laskemalla B-vitamiinin ja sen johdannaisten tasoja. Halutuista tuloksista riippuen sopivan fermentointitekniikan valinta on oleellista. Tällöin tulevaisuudessa lupiinista voitaisiin tuottaa proteiinipitoisia vaihtoehtoja eläinperäisille maitotuotteille.

Ympäristöllisistä ja terveydellisistä syistä kasvipohjaisten tuotteiden, erityisesti palkokasvien, vihannesten, marjojen ja hedelmien, määrää ravinnossa tulisi lisätä. Kasvipohjaisilla tuotteilla on yleisesti ottaen hyödyllisiä vaikutuksia, mutta eroteltuihin kasvipohjaisiin raaka-aineisiin perustuvien tuotteiden vaikutusta terveyteen ei vielä täysin ymmärretä. Kasvipohjaiset tuotteet vaativat yleensä jonkinasteista prosessointia ja sen vaikutusta niiden biokemialliseen koostumukseen tulee tutkia vielä syvällisemmin. Kun puhutaan kasvipohjaisten proteiinirikkaiden tuotteiden terveellisyydestä olisi tärkeää ottaa huomioon ravintoaineiden ja muiden biokemiallisten yhdisteiden määrä ja laatu mahdollisten luokittelusysteemien kategorioiden sijaan, koska kasvipohjaiset tuotteet voidaan usein sijoittaa terveydelle haitallisena pidettyyn ”ultraprosessoitu” kategoriaan. Tämä väitöskirja tuo tärkeää tietoa näihin kysymyksiin, erityisesti keskittyen fermentoinnin hyödyntämiseen kehitettäessä kasvipohjaisia proteiinipitoisia tuotteita.

LIST OF ABBREVIATIONS

AAB	Acetic acid bacteria
HILIC	Hydrophilic interaction
ILA	Indolelactic acid
LAB	Lactic acid bacteria
LDL	Low-density lipoprotein
LC	Liquid chromatography
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
PBPR	Plant-based protein-rich
PBD	Plant-based diet
PCA	Principal component analysis
PLA	Phenyllactic acid
QTOF	Quadrupole time-of-flight
RP	Reversed phase
SCOBY	Symbiotic culture of bacteria and yeast
UHPLC	Ultra high-performance liquid chromatography

LIST OF ORIGINAL PUBLICATIONS

I. Raita, J.; Ahmed, H.; Chen, K.; Houttu, V.; Haikonen, R.; Kårlund, A.; Kortesniemi, M.; Yang, B.; Koistinen, V.; Hanhineva, K. Existing food processing classifications overlook the phytochemical composition of processed plant-based protein-rich foods. *Nat Food.* **2025**, 6, 503-512.

II. Raita, J.; Ahmed, H.; Nikola, M.; Haikonen, R.; Kelanne, N.; Laaksonen, O.; Yang, B.; Koistinen, V.; Hanhineva, K. Differences in metabolic profiles of lupin-based beverages produced with various fermentation techniques. *Accepted.*

1 INTRODUCTION

As the global population continues to grow, so does the demand for increased food production and processing. Livestock production in agriculture is a major contributor to greenhouse gas emissions, and the following food processing is environmentally burdening, contributing to climate change[1–3]. Furthermore, Western diets which are often low in *e.g.* dietary fiber and bioactive compounds and high in highly processed meat products, are known to increase the risk of chronic diseases and obesity[4,5]. To address these challenges, changes in dietary patterns are necessary to provide sustainable and healthy food for the global population. The focus should be on the production of vegetables, fruits, and nuts to ensure a sufficient protein supply, alongside animal-based sources, which can be an important part of a nutritionally adequate diet.[1,6] As such, promoting dietary patterns focusing on protein-rich plant-based foods such as soy, peas, and whole grains, which can be adapted to regional conditions, is crucial. These foods are not only associated with health benefits but can also be produced with more environmentally sustainable methods[3,7].

The health-beneficial aspect of diets rich in plant-based foods is evidenced *e.g.* by the fact that people consuming plant-based diets (BPD) have a decreased risk for cardiovascular diseases, as has been demonstrated in numerous epidemiological studies[8–10]. Health benefits from plant products derive from *e.g.* unsaturated fats, protein, dietary fiber, phytochemicals, vitamins, and minerals[11], although molecular mechanisms behind their beneficial effects are not fully understood[12]. Plant phytochemicals, *e.g.* flavonoids and phenolic acids, are known for their health-promoting benefits such as anti-inflammatory and antioxidative properties[13,14]. To date, food and nutrition research has typically focused on a narrow range of nutrients, even single compounds, and the comprehensive profiling across the wide polarity scope of the thousands of biochemicals present in any food has not been disclosed. The current understanding of the health implications of food is based on <200 basic nutrients, even when foods contain thousands of biochemicals that may exert bioactive potential, and this pool of compounds should be more carefully assessed[12].

Plant-based protein-rich (PBPR) foods, which are often prepared through various processing techniques, have become extremely popular during the past few years. These products are commonly made from soy, peas, and wheat, and usually contain a mixture of different plant protein extracts to achieve better amino acid composition or to improve organoleptic properties. Processing techniques vary from drying to heavily refined products containing only the extracted protein part of the plant. Heavily refined products may therefore have poorer nutritional composition compared to the raw material, due to the loss of nutritionally important components during processing, such as fiber and

bioactive compounds. Furthermore, foods made from heavily refined products also typically include high levels of added sodium[15], and therefore there is an urgent need to better elucidate the biochemical composition and related health implications of PBPR foods produced with various processing techniques.

Fermentation with live microorganisms is one of the most traditional food processing methods across the globe and is of interest because of its ability to modify the chemical composition of the food raw material. It can be used to preserve food and enhance its digestibility, as well as provide unique textures and flavors, and remove antinutrients. Fermentation can also facilitate the absorbability of plant matrix-bound phytochemicals and cause the formation of new, potentially health-promoting bioactive compounds from plant-based materials[16].

In this thesis, we used a liquid chromatography – mass spectrometry (LC–MS) -based metabolomics approach to address the impact of various processing techniques on PBPR foods, which closely relates to the challenges regarding healthiness of heavily processed plant-based foods. We analysed the impact of processing on the biochemical composition of commercial plant-based foods by choosing products made with varying processing techniques, raw materials, and from different food categories, such as whole plant foods and products made from protein concentrates/isolates. In addition, we tested four different fermentation techniques with lupin-based beverages to study the changes that occur during fermentation and due to different fermentation cultures.

The results obtained in this thesis regarding the biochemical composition of commercial plant-based foods and fermented lupin-based beverages offer valuable information for assessing the healthiness of these plant-based foods, and for their further development. In addition, these results can be used to improve and develop food processing classification systems to guide consumers towards healthier dietary choices.

2 REVIEW OF THE LITERATURE

2.1 Plant-based protein-rich foods

PBD consisting of foods such as whole grains, legumes, and berries, has been associated with several health benefits[17]. For instance, replacing animal-based proteins with plant-based ones can improve the plasma lipoprotein profile by decreasing the content of low-density lipoprotein (LDL) and total cholesterol[18]. Furthermore, PBD consisting of vegetables, fruits, and seeds was found to decrease systolic and diastolic blood pressure after four weeks of consumption. In addition, other beneficial health effects *e.g.* reduced weight, waist circumference, and insulin have been observed[19]. The impact of PBD on the prevention and treatment of type 2 diabetes has been previously acknowledged[20], and whole food PBD together with exercising can improve glycaemic control and possibly reduce the need for both diabetes and cardiovascular medicine[21]. In addition, healthy PBD was found to be associated with lower risk for cardiovascular diseases and all-cause mortality[22].

Plant-based foods vary from whole foods containing the whole plant material, *e.g.* whole beans, to more processed foods made from protein concentrates or isolates extracted from the plant material. Often, these products are made from plant-based materials such as legumes, grains, vegetables, nuts, and seeds. Currently, PBPR foods are gaining attention as new products made from various raw materials are being developed. These products include *e.g.* burger steaks, minced and pulled products, and extruded chunks. PBPR foods are often made to resemble meat and produced from extracted plant components, such as proteins. Compared to animal-based proteins, plant-based ones may have limited functional properties, like viscosity and emulsification[23]. Foods made from plant-based raw materials with different processing techniques can be associated with undesirable textural properties or off-flavors, which can impact their acceptance by consumers[24]. Therefore, they might contain various additional ingredients to improve these issues.

Regarding the nutritional content of PBPR foods, one important aspect is that they usually have added micronutrients to match the ones present in animal-based foods[25–27]. However, PBPR foods might still contain inadequate amounts of zinc, iron, and vitamin B12 as examples[28,29]. While some PBPR foods may contain similar amounts of iron as animal-based foods, it is often present in a different form which might have lower bioavailability for humans[28]. Still, a clear advantage when comparing plant-based foods with animal-based ones is the high content of dietary fiber present in PBPR foods[30], along with other bioactive compounds, such as polyphenols[31]. However, one of the concerns regarding the

nutritional quality of PBPR foods is that they can often have a high content of sodium[25,30,32].

Another popular form of plant-based foods are plant-based beverages. These water extracts are often made from various grains, nuts, seeds, and legumes. The reason for their popularity rises from the need for more environmentally sustainable alternatives for cow's milk and the allergies associated with it[33,34]. As expected, lactose is one compound differentiating animal-based milk from its plant-based alternatives, but other compounds, such as trigonelline and theophylline, can be found specifically in plant-based beverages[35]. In addition, plant-based beverages contain more Maillard reaction products resulting from lower content of creatine in comparison to animal-based milks[36,37]. In relation to other nutritional factors, plant-based alternatives have been found to contain lower quantities of essential amino acids and their protein and carbohydrate contents are dependent on the used plant material[37]. Similarly, with PBPR foods, plant-based beverages are often fortified with micronutrients, such as vitamin B12 and calcium, to improve their nutritional quality. In case of unfortified plant-based beverages, the intake of those micronutrients can be drastically less than it would be when compared to consuming the animal-based option[38].

2.1.1 Raw materials in processed plant-based protein-rich foods

One of the most popular raw materials used in PBPR foods are soybeans (*Glycine max*) owing to their high content of protein. In addition to soy, another widely used raw material are peas (*Pisum sativum*), which have similar functional properties as soybeans *e.g.* emulsifying and water-holding properties[39,40]. They can be used to prepare products often associated with soy, such as tofu and tempe[41]. From grains, wheat (*Triticum aestivum*) protein is a popular raw material in plant-based foods due to its water absorbent properties and the capability to form meat resembling fibrous structures, which are formed during kneading in the preparation of seitan products. However, the content of essential amino acid lysine can be low in wheat protein[42]. PBPR foods often combine various plant-based raw materials to improve the nutritional quality, including protein content and amino acid compositions.[25,43–45] Additional plant-based sources that could also be cultivated in Northern latitudes including Finland, such as fava beans (*Vicia faba*) and narrow-leaved lupins (*Lupinus angustifolius*), have recently gained attention.

2.1.1.1 Soy

Soybeans are high in protein and their protein content in dry seeds is approximately 40 % consisting mostly of globulins. Therefore, they are widely

used in food products and also in animal feed.[46] However, there are also downsides to soy, and one of them is its allergenicity, which is an important aspect to consider when developing PBPR foods. Usually it has been associated with glycinin and β-conglycinin, which are proteins present in soybeans.[47] Moreover, another downside with soy production is that its cultivation in Europe can be challenging due to growing conditions, thus making it non-sustainable[48].

One of the most well-known soy-based foods is tofu, which could also be described as soy curd. It is made from soybeans by extracting and coagulating proteins (**Figure 1**). The traditional method of tofu preparation includes washing, soaking, and grinding the whole beans before filtering it to produce a milk-like beverage. After that follows heating of the beverage, adding the coagulant solution, and finally pressing the tofu into the desired form.[46] Based on the composition of the product, tofu's can be divided into soft or firm ones.

Usually a lot of soy-based foods are made from soy protein concentrates or isolates. Additionally, extrusion is often employed in the production of soy-based foods. Extruded products can be prepared from *e.g.* flour or from the extracted proteins. However, foods like tempe are made from whole soybeans. (**Figure 1**)

Soybeans contain various bioactive compounds like isoflavonoids, which can have beneficial health effects. For instance, soy proteins have been associated with reduced serum cholesterol[49] and soy oil with reduced risk for coronary heart disease[50]. Also, fermented soy products have been found to have beneficial health effects, such as possible cognitive improvements in older women[51] and reduced risk for osteoporosis[52].

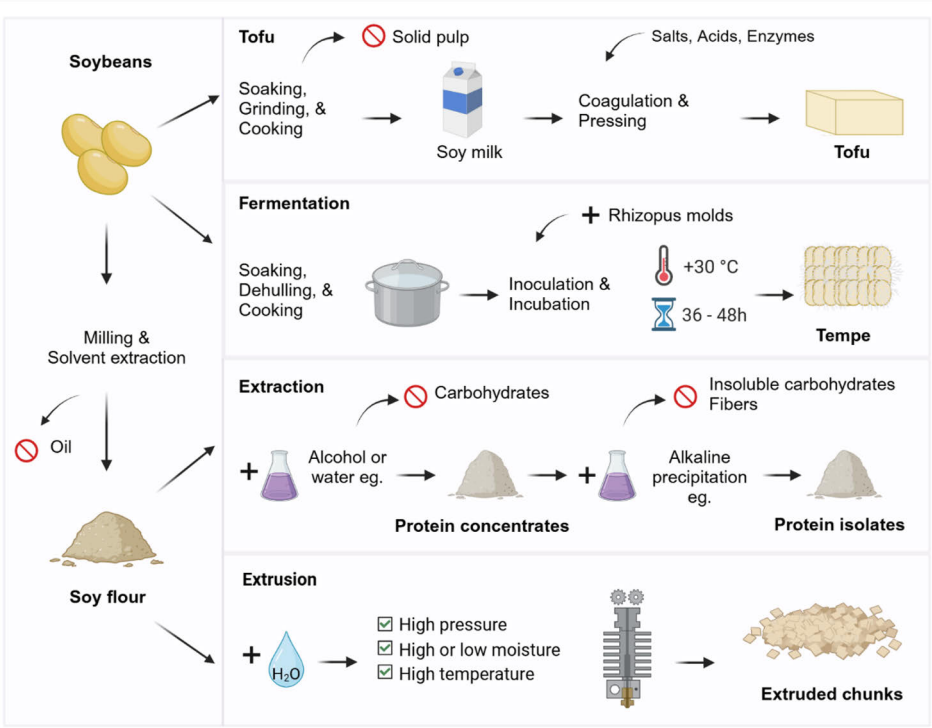


Figure 1. Processing techniques for soybeans to demonstrate the preparation of tofu, tempe, extraction of protein concentrates or isolates, and extruded chunks. Figure modified from Raita, et al. (2025)[53].

2.1.1.2 Lupin

Lupins are legumes with a high content of protein and fiber, and they can be grown in Europe, including Finland. Therefore, they could work as a more sustainable option to soy, which needs to be imported.[54,55] The Lupinus genus consists of various species, but only some of them are safe for consumption due to their antinutrients, such as alkaloids. Edible species include narrow-leaved lupins (*Lupinus angustifolius*), white lupin (*Lupinus albus*), yellow lupin (*Lupinus luteus*), and tarwi (*Lupinus mutabilis*). However, even these edible species differ in their alkaloid contents[54,56,57]. These alkaloids are often water-soluble, and thereby their quantities can be reduced with debittering processes, including water soaking[56].

In addition to their high content of protein and fiber, lupins can contain vitamin B compounds and various phytochemicals, and they are rich in essential amino acids and unsaturated fatty acids[55,57]. Depending on the lupin species, they can have different chemical compositions. For instance, yellow lupins were found to contain less non-starch polysaccharides when compared to white and narrow-leaved lupins[58]. Differences also exist between cultivars, as some of them can contain more phenolic compounds, saponins, phytic acid, and quinolizidine

alkaloids[55]. However, fermentation can be used to decrease the content of antinutrients, *e.g.* phytic acid, as some microbial strains are able to produce phytase which can result in the degradation of phytic acid, in addition to the impact of lower pH[59,60]. When developing products from lupins, it would be essential to choose the most suitable species and cultivars to ensure proper nutritional composition. In addition, lupins could be added to food products to increase their nutritional value. This was demonstrated by Elhordoy et al.[61], as they produced extruded meat alternatives with lupin flour and found that the final products contained compounds with antioxidant properties.

Many of the beneficial effects behind lupins stem from the bioactive compounds present in them. Consumption of lupins has been associated with health benefits, such as prevention of hyperglycaemia, hypertension, and dyslipidemia[62]. Intake of lupin protein isolate or concentrate could also reduce LDL cholesterol[63,64]. However, varying results exist on the health outcomes after lupin consumption and these results are dependent on whether whole lupins or isolated proteins were used.[65]

2.1.2 Phytochemicals in plant-based protein-rich foods

Phytochemicals are plant-derived compounds with bioactive properties. They are also referred to as secondary metabolites, which plants use for adaptation to the environment *e.g.* defense against insects[66]. Phytochemicals consist of several compound classes, such as polyphenols, alkaloids, and terpenoids. Because phytochemicals differ widely in chemical structures, their bio accessibility is highly dependent on the food matrix, and they can have limited bioavailability due to their poor solubility and stability[67,68]. Therefore, even when high concentrations of phytochemicals are present in foods, their bioavailability might not be directly correlated with quantity[69], however it can be affected by food processing[70]. For instance, malting process can be used to increase the content of phytochemicals[71] and fermentation is known to improve the bioavailability of isoflavonoids[72]. Additionally, cooking and storage conditions can have significant impacts on phytochemicals, as was shown in a study where boiling and freezing of broccoli decreased the content of these compounds along with vitamin C[73].

Phytochemicals can be obtained through diet from plant-based foods including fruits, vegetables, nuts, and legumes. In addition, whole grains are one important source for phytochemicals[71]. The content of phytochemicals varies based on the raw material[74]. Some examples of dietary phytochemicals include lycopene obtained from tomatoes[75], ferulic acid from maize[76], and caffeic acid from coffee[77]. In general, dietary requirements for phytochemicals have not been defined, but seasonally adjusted diets consisting of various plant-based whole

foods and drinks like tea and coffee, can provide from 1441 to 1607 mg of phytochemicals in total daily[78]. However, for flavan-3-ols the intake of 400-600 mg daily has been recommended for cardiometabolic protection[79]. For polyphenols, which are one of the most abundant and important group of phytochemicals in plant-based foods, the estimated daily quantities can vary from 536 mg to 750 mg in Latin America[80] and from 744 mg up to 1786 mg in Europe[81]. Intake of polyphenols varies greatly depending on the consumed diet, for instance higher consumption of tea, coffee, and red wine result in higher intakes[82].

Flavonoids, which are widely present in plants, are an important class of phytochemicals belonging to polyphenols. Fruits and vegetables are great sources to obtain these compounds from the diet[83]. Their structural characteristics include two aromatic rings and one heterocyclic ring containing oxygen. Based on the oxidation level of the heterocyclic ring, they can be further divided into groups, such as isoflavonoids, flavones, and flavonols. In nature, these compounds are often present as sugar conjugates, which improve their stability.[84] However, some processing techniques can modify the substitutions of these compounds. For instance, fermentation can increase the content of aglycone forms which do not contain the sugar conjugates[53]. These aglycone forms are known to have higher bioavailability for humans[72]. In addition, dietary intake of flavonoids has been associated with lower risk for type 2 diabetes, cardiovascular diseases, and all-cause mortality[85].

Isoflavonoids, also referred to as phytoestrogens, are often found in legumes of which soy is the main source for these compounds[86]. Main isoflavonoids found in soybeans are genistein, daidzein, glycitein, and their different conjugates (**Figure 2**)[87]. For instance, they participate in plant defense and growth[88]. Their chemical structure differs from other flavonoids by the position of the phenyl ring substitute. After consumption of isoflavonoid-rich foods, these compounds are metabolized by the gut microbiota to aglycones before they can be absorbed[89]. However, they can also be further metabolized, for instance genistein can be converted into *p*-ethylphenol and daidzein into equol[90]. Equol has been associated with decreasing cardiovascular biomarkers and also menopausal symptoms[91]. The reason why these compounds are also referred to as phytoestrogens, is that they have similar structures with estrogenic hormones and can thereby interact with estrogen receptors[92]. Isoflavonoids have been associated with antioxidant properties[93], anti-inflammatory effects[94], bone health for postmenopausal women[95], and reduced risk for breast cancer[96]. Their use as an alternative for hormone replacement therapy in menopause has also been investigated and found to be efficient[97].

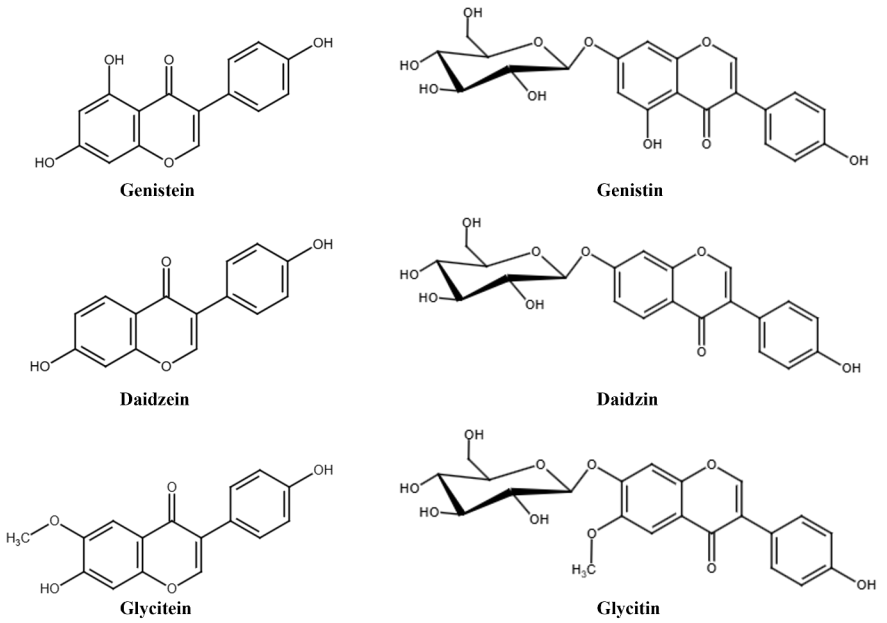


Figure 2. Chemical structures of isoflavonoids genistein, daidzein, glycitein, and their sugar conjugates genistin, daidzin, and glycitin.

Alkaloids are a diverse group of phytochemicals in plants, but they are also found in animals. They can contain one or several nitrogen atoms depending on their type and they are derived from amino acids[98,99]. Relatively low doses of alkaloids present in many plants can have toxic effects on humans and animals after consumption. Pyrrolizidine alkaloids are one group of alkaloids, and their consumption can lead to death in the worst-case scenario. Acute consumption can lead to liver damage or failure, but they can also accumulate on tissue and cause long-term damage[100,101]. Quinolizidine alkaloids, including lupanine, 13-hydroxylupanine, angustifoline, and sparteine, are the main alkaloids found in lupins[102] (**Figure 3**). In addition to pyrrolizidine alkaloids, also the consumption of quinolizidine alkaloids have been associated with risks for humans[103]. However, some plant alkaloids can have beneficial effects and they have been used as medicinal components, for instance vincristine has been used as a chemotherapeutic drug[104,105]. Though, with a proper debittering process, the content of alkaloids can de reduced to safe limits[106].

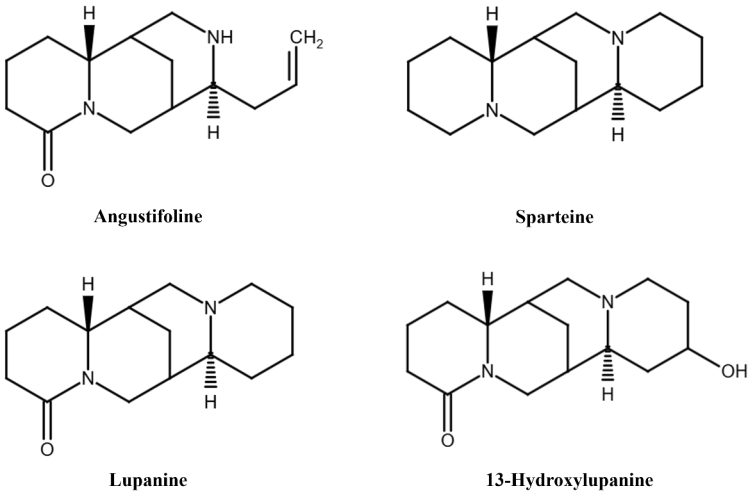


Figure 3. Chemical structures angustifoline, sparteine, lupanine, and 13-hydroxylupanine which are commonly found alkaloids in lupins.

Phenolic acids, for example obtained from coffee and whole grains[83], are divided into hydroxycinnamic and hydroxybenzoic acids and exist in all plant parts[107]. Common phenolic acids include caffeic acid, *p*-coumaric acid, chlorogenic acid, and cinnamic acid[108,109]. They are known to have antioxidant and antimicrobial properties, but higher number of hydroxyl or methoxy groups in the structure can result in decreased antimicrobial properties[110]. Phenolic acids have good bioavailability, but fermentation can increase it with these compounds too by increasing the content of their free forms[111].

2.1.3 Amino acid composition of plant-based protein-rich foods

Among the main concerns regarding the nutritional quality of PBPR foods are their protein content and whether they contain all of the essential amino acids. Plant proteins can lack some of the essential amino acids and they might also have low protein digestibility[112]. The information regarding the protein composition and its quality in plant-based foods is scarce, as it is dependent on the individual product type analysed or consumed, as well as the information regarding whether it has been prepared from single plant protein source or combined from several ones.

However, some of the concerns regarding the digestibility of plant-based proteins can be tackled with food processing[112]. Yang et al.[30] found out that the total protein content in plant-based foods was at least adequate based on the required protein amount for adults, but the amino acid profile can be misbalanced compared to animal-based one. Nevertheless, in another study by Harnack et al.[28] most of the plant-based products made to replace ground beef were found to contain considerably less protein than the animal-based counterparts. Sometimes

processing can have a negative impact on the nutritional quality of amino acids; for example treatment with high temperatures can induce changes in the side chains. In addition, various reactions can occur during storage, which might also negatively impact their nutritional quality. One of such reactions is Maillard reaction, which can have a significant impact on the essential amino acids present in foods.[113]

Limiting amino acids in plant-based foods have often been found to be lysine[114] and methionine[115]. However, quinoa for instance contains higher levels of lysine and methionine[116]. This highlights the importance of combining various plant-based raw materials to ensure the appropriate amino acid composition as wheat-, soy-, and pea-based products can be low in these essential amino acids.

Xie et al.[117] studied the digestibility of pork, beef, and their plant-based alternatives using an *in vitro* digestion model. The authors found out that there was a significant difference between the protein digestibility based on the simulated gastrointestinal digestion model, as plant-based alternatives had lower digestibility in the gastrointestinal phase compared to their animal counterparts. One reason for the lower digestion of plant-based proteins might be the high content of starch. In addition, animal-based products released more bioactive peptides during the digestion. Similarly, Cutroneo et al.[118] stated that based on the INFOGEST *in vitro* model they used, animal-based burgers had slightly higher protein digestibility than plant-based ones. For plant-based yogurts, Zeinatulina et al.[119] found that the *in vitro* digestibility was approximately 80% meaning, that majority of the proteins present in the yogurts were broken down during the digestion and could thereby possibly be absorbed.

2.1.4 Antinutrients in plant-based protein-rich foods

Antinutrients in PBPR foods are compounds that can have negative health effects, such as inhibition of mineral absorption, for humans after their consumption. Some common antinutrients present in plant-based foods are proteins like lectins and trypsin inhibitors, phytochemicals like tannins, phytates, and saponins, and raffinose family oligosaccharides[120].

Lectins, highly present in beans and peas, are proteins which can have an impact on the absorption of amino acids. These compounds are produced by plants for protection against insects. In animal studies they have showed adverse health effects, but their quantities can be reduced by soaking or boiling the beans before consumption[121]. For humans, lectins can cause clumping of red blood cells, and symptoms of lectin poisoning include vomiting and diarrhea.[113] Trypsin inhibitors on the other hand can impact the digestibility of plant-based proteins, and therefore they are preferably removed from the food material[122]. However, processing techniques such as extrusion, heating, microwaves, or wet-

preconditioning, were found to decrease the levels of trypsin inhibitors and tannins in legumes[123,124]. Plant-based foods can contain high quantities of phytic acid, which can act as an inhibitor for absorption of minerals, such as zinc and iron[125]. In a study conducted in Sweden, the content of phytate was analysed in commercially available meat alternative products. Soy, wheat, and pea proteins were the main ingredients in those products, but some products were made from whole beans (chickpeas, farm beans, and white beans), mycoprotein, and fermented peas. Highest contents of phytate were found in foods prepared from whole beans and soy, pea, and wheat protein. Protein extraction can accumulate phytate which might explain this observation[29].

During fermentation of PBPR foods, biogenic amines can be formed through decarboxylation of amino acids or through amination and transamination of aldehydes and ketones[126,127]. Various microorganisms are capable of producing these compounds resulting in their accumulation in the food product[128]. These biogenic amines include compounds like tyramine, histamine, spermidine, and putrescine, which can have adverse effects on human health[129,130]. For instance, their high consumption can lead to an increase in blood pressure and blood sugar levels, migraine, hypotension, and release of adrenaline and noradrenaline[128]. Tyramine has been recognised as one of the most toxic compounds in the group of biogenic amines, as it can have negative effects if consumed together with monoamine oxidase inhibitor (MAOI) antidepressant drugs by increasing blood pressure. The levels of tyramine in foods vary based on the food type, and deaths related to excess consumption of tyramine with MAOI drugs are rare.[131] With spermidine, conflicting literature exists as it has been associated for instance with possible beneficial impact on aging[132]. Fish products, beverages like wine and beer, and fermented foods including plant- and animal-based products are known to contain highest levels of biogenic amines[133]. However, by selecting specific starter cultures, appropriate fermentation temperatures, using food preservatives and processing methods like irradiation, their formation can be controlled[133,134].

2.2 Processing techniques for plant-based foods

In general, foods typically go through varying processing steps on their way from primary production to ready dishes. Food processing includes all kinds of alterations made to the product, whether it happens in industry, home kitchen, or restaurants. The main reasons for processing include improving shelf life, nutritional quality, safety, or sensory properties making the food products more appealing. Processing techniques include everything from drying and peeling to more intrusive approaches, such as boiling, frying, and extracting specific components[135]. This also applies to plant-based foods, as they are often processed in some ways. These processing techniques can vary from drying or preserving

the whole plant material to extracting components such as protein, oil, and sugar. Commonly used processing techniques for plant-based foods include extrusion, fermentation, protein extraction, and preservation. PBPR foods often go through various processing techniques to achieve the desired textural properties, while products made from whole plant materials might require less.

During food processing, some of the beneficial compounds present in the plant material can be lost[53,136,137]. Even though plant-based foods are often perceived as healthier alternatives to meat, due to some intrusive processing techniques and added ingredients this might not always be the case[138].

2.2.1 Extrusion

Extrusion is a common food processing technique used to produce foods with modified textural properties such as fibrous structures[139]. For plant-based foods, extrusion is a suitable method for producing texturized proteins that can be further utilized in the production of meat alternatives. While the food mass moves through the extruder, proteins start to denaturate forming fibrous structures before the mass exits the extruder. Based on the applied moisture content within the die, either low- or high-moisture products can be formed.[23] With low-moisture extrusion, a dry product with a puffed texture can be produced[139,140], while with high-moisture extrusion products with meat like fibrous structures can be produced by cooling the mass before it exits the extruder[141].

Parameter optimization and raw material selection for extrusion are crucial, as they have major impacts on the textural properties of the product. Moisture content and high temperatures can impact the sensory properties by modifying viscosity of the material due to protein denaturation and starch gelatinization[142–144]. When high temperatures are applied, the content of microorganisms in the product can be reduced to improve the safety of the food, and additionally some naturally present enzymes can be inactivated.[145] In addition, modifying the pH of the raw material can also result in an improved texture of the product[146]. In a study by Lazou et al.[147], they found that increasing the moisture content resulted in products with harder textures, while moderate temperatures and moisture content were found to be optimal for producing snacks with acceptable textures. Likewise, Osen et al.[148] concluded that textural properties can be modified with the temperatures used during extrusion. Extrusion has also been shown to increase the total phenolic content of cereal and legume by-products[149].

2.2.2 Fermentation

Fermentation is an ancient food processing method where microorganisms are used to preserve food and improve nutritional properties by modifying its components. For instance, these microorganisms are able to modify the composition of proteins, carbohydrates, and fats. Depending on the fermentation type, they can be either already present in the fermented material or added later on. Fermentation cultures can be selected based on the desired attributes, while spontaneous fermentation is based on the microorganisms already present in the raw material or the fermentation environment. Commonly used microorganisms in fermentation include yeasts and lactic acid bacteria.[150] For plant-based foods, fermentation can be applied to produce foods such as tempe, kimchi, natto, yogurts, and soy sauce. Tempe originates from Indonesia and is made by fermenting the whole beans with *Rhizopus* spp. molds resulting in a solid cake type of a product covered by the mold[151]. Soybeans are commonly used in the preparation of tempe, but other raw materials like faba beans can also be used[152].

With plant-based foods, fermentation is often used to reduce the amount of antinutrients, improve sensory properties and improve preservation. Fermentation can influence the bioavailability of some phytochemicals by releasing them from the plant matrix and thereby increasing the amount of their free forms[153]. For instance, during fermentation the β-glucosidase activity of the microbes can increase the amount of aglycone forms[72,154,155]. Nutritional availability of other components can also increase, as the microorganisms present in the fermented material can predigest them into more bioavailable forms.[125]

2.2.2.1 Lactic acid bacteria fermentation

Lactic acid bacteria (LAB) have been used for the preparation of dairy products as well as plant-based foods in industry and home kitchens for various purposes. LAB fermented foods worldwide include *e.g.* bread products like sour bread and rye bread from Europe, alcoholic beverages and fermented cereal products in Africa, and commonly recognized products like kimchi, sauerkraut, and salami[156]. LAB are classified as gram-positive bacteria relating to the thickness of their cell walls. Depending on the species, they can be homo- or heterofermentative. Homofermentative species mainly produce lactic acid from fermentation of sugars and in addition to lactic acid, while heterofermentative LAB produce other end products *e.g.* acetate, ethanol, and carbon dioxide. In addition to the impact of species on these end products, fermentation conditions can also have an impact.[157,158] LAB fermentation can be used to improve the shelf life and sensory properties of foods, as the production of organic acids acidifies the fermented material thus improving these aspects.[159] Example of a LAB

species commonly found in nature is *Lactiplantibacillus plantarum*, previously known as *Lactobacillus plantarum*. This species is considered safe to be used in food applications, depending on the strains[160].

Fermentation with LAB can be used to modify the nutritional quality and sensory properties of plant-based foods[161]. With plant-based juices, bioavailability of phenolic compounds and vitamin B2 were found to improve after fermentation with LAB[162]. In addition, LAB fermentation can modify sensory properties of lupin beverages, and the fermented option was preferred over the unfermented one[163]. In case of LAB fermented plant-based alternatives to salami and other sausage products, the composition of the plant-based raw material, including amino acids, lipids, and carbohydrates, can provide challenges as those components are important aroma precursors for characteristics associated with the animal-based options[164]. However, LAB fermentation is still a feasible method to improve aroma profile of plant-based protein extracts[165].

2.2.2.2 Spontaneous fermentation

Spontaneous fermentation is based on the presence of microorganisms in the fermented raw material, equipment, or environment. It is a traditionally used fermentation method for the production of various food products and beverages, including kimchi, olives, and sauerkraut.

Because spontaneous fermentation happens under uncontrolled conditions and without a defined starter culture, it can have unpredictable results. For instance, it might lead to the growth of unwanted bacteria possibly deteriorating safety of the product. In industry, fermentation conditions like temperature can be controlled to produce more reproducible products, or starters from previous spontaneously fermented batches can be inoculated in to the product for a more time-efficient production[166]. During uncontrolled spontaneous fermentations toxic compounds can be formed due to the presence of unknown and unwanted bacteria or yeast. However, this problem exists with all fermentation techniques, but is more prominent with spontaneous one due to the uncontrolled conditions.[167,168]

Besides the possible negative impacts of spontaneous fermentation, it can result in positive effects like the reduction of anti-nutrients[169]. For instance, reduction of phytic acid in grains and legumes after spontaneous fermentation has been reported by various studies[170–172]. Likewise in other fermentations, the content of bioactive compounds can increase. Content of total flavonoids and phenolic compounds has been shown to increase during spontaneous fermentation as they are released from the plant matrix promoting its health beneficial effects[173].

2.2.2.3 Kombucha fermentation

So far, kombucha fermentation has not been extensively used in the production of PBPR beverages. However, some studies where kombucha beverages have been prepared from soy-based beverages have been conducted[174,175]. The starter culture used in kombucha fermentations is a symbiotic culture of bacteria and yeast (SCOBY). Traditionally kombucha is made by fermenting sweetened tea with SCOBY, but other liquid raw materials can be fermented in the same way. SCOBY is a biofilm which forms on the surface of the liquid due to the interactions between the bacteria and yeasts under static conditions. During the kombucha fermentation, new layers of this biofilm are formed and they can be used to produce new batches of kombucha by transferring them to the sweetened liquid material.[176] The main contributors to the formation of this biofilm are acetic acid bacteria (AAB), which are commonly found in kombucha starters. In addition, LAB are also present in kombucha.[177–179] However, the employed tea material has an impact on the microbial composition of the kombucha beverage, as green tea appears to favor LAB whereas AAB prefer black tea[180]. Fermentation temperatures can also have an impact on the microbial composition of kombucha, which can lead to differences in the abundances of some organic acids like glucuronic acid[181].

Kombucha beverages contain beneficial compounds, such as polyphenols and organic compounds with antioxidant properties[182,183]. In addition, kombucha has antimicrobial properties[184]. Some of the beneficial effects of kombucha are related to the phytochemicals present in the tea, but also due to the chemical changes in the composition during the fermentation process.[185] In a study by Mendelson et al.[186], they found that kombucha consumption was associated with lower fasting blood glucose levels for type 2 diabetes patients. Furthermore, regular consumption of kombucha can influence the gut microbiota positively[187]. However, there has been debate regarding the possible toxic effects of kombucha consumption. In some cases, high consumption has led to liver damage[188].

2.2.3 Protein extraction

Protein concentrates or isolates derived from the plant material are often used when manufacturing more processed plant-based foods, such as burger steaks and minced or pulled products. For this purpose, the proteins need to be extracted by removing other components *e.g.* carbohydrates, fiber, and lipids. This is often done using dry or wet extraction, which both include several steps before obtaining the protein concentrate or isolate. Usually, wet extraction techniques are used to produce protein isolates, while dry extraction methods are used to produce protein concentrates. Food industries often employ wet extraction

methods. However, large amounts of water are needed for this technique thus impacting its environmental load.[189,190]

Protein concentrates are prepared after oil removal by removing the remaining soluble carbohydrates, while protein isolates are produced by removing the remaining insoluble carbohydrates and dietary fiber, which results in a higher protein content.[191] The applied extraction method has an impact on the protein content of the extract, as well as on the solubility, water and oil holding capacities, and foaming and emulsifying properties of the proteins[192]. Therefore, choosing the right extraction method can also have a positive impact on the textural properties of the product.

During protein extraction, some bioactive compounds can be lost when removing other components from the plant material. PBPR foods made with protein concentrates or isolates can contain lower abundances of phytochemicals compared to products made with whole beans or without protein extraction[53]. For instance, water-soluble compounds like tannins can be lost during wet extraction[193]. In a study comparing pea flour with pea protein isolate, the content of phenolic compounds and flavonols were lower in the protein isolate, but on the other hand the content of anthocyanins was higher. In the same study white bean flour and its protein isolate were compared, and the results were opposite as flavonols and total phenols were higher in the isolate, while anthocyanins were lower.[194] The final content of bioactive compounds in protein isolates or concentrates is highly dependent on the extraction method and raw material. As an example, the degree of pH decreasing during protein extraction can have an impact on the total phenols in the protein isolate, as sharper decrease leads to higher content than decreasing the pH more steadily[195].

2.2.4 Food processing classification systems

Food processing classification systems have been developed and used to aid consumers in making healthier dietary choices. These systems aim to determine the impact of various processing techniques on food products, as some of the more processed products have been associated with adverse health effects in epidemiological studies. During various processing techniques, the nutritional content of the raw material can change either positively or negatively. Literature regarding the impact of individual processing techniques on the nutritional components of foods exists, but for instance there is not enough information regarding plant-based foods which often go through several processing steps.

Food processing classification systems based on the degree of processing include NOVA[196], IARC-EPIC[197], IFPRI[198], Poti et al.,[199], and IFIC[200] as examples (**Figure 4**). Some classification systems are based more on the nutritional aspects, rather than on food processing techniques. These classifications include for instance Nutri-Score, which has been used as a front-

of-packaging labelling in France and other European countries[201]. In Nutri-Score, foods receive scoring from A to E depending on the nutrient content including energy, total sugar, saturated fatty acids, sodium, protein, fiber, and content of fruits, vegetables and nuts. Foods with A have the highest nutritional quality while E graded foods have the lowest[202].

The existing systems, such as NOVA, have been criticized for insufficient consideration of the science behind food processing and for confusing definitions for the classifications[203,204]. Another issue with these systems is that they have, perhaps unintentionally and jointly with effort from the media, slightly demonized the concept of food processing for normal consumers. Food processing is often necessary and can result in positive outcomes, but the negative media attention concerning "ultra-processed" foods has brought a negative nuance towards food processing itself, even though ultra-processing does not exist as a food processing technique as such. Moreover, none of the existing classification systems consider the comprehensive biochemical composition of foods. These systems often consider what has been added to the product, but not what has been removed from the raw material during processing.

Recently, so called ultra-processed foods have gained a lot of attention, as they have been associated with obesity[205], higher risk of mortality[206], type 2 diabetes, and cardiovascular diseases[207] in several epidemiological studies. There is no clear evidence yet what is the reason behind these adverse health effects in ultra-processed foods, but these foods are often calorie-dense and contain high amounts of sugar, saturated fat, and sodium[208,209]. Other proposed factors might be the added ingredients, such as emulsifiers, artificial sweeteners, and other preservatives[210]. Each of the previously mentioned factors relate to added ingredients and nutritional components in the food, rather than on the impact of the actual processing technique.

2.2.4.1 NOVA classification system

The NOVA[196,211] food processing classification system categorises foods into four different groups; 1. Unprocessed and minimally processed foods, 2. Processed culinary ingredients, 3. Processed foods, and 4. Ultra-processed foods. Group 1 consists of edible parts of plants or animals without addition of other substances, such as salt or sugar. Processing techniques include for example freezing, peeling, drying, and boiling, and these processes aim to extend the life of the product and remove the inedible parts. Example foods include eggs, flour, yoghurt without added sweeteners or sugar, and meat. Group 2 consists of combinations of components obtained from group 1 foods. Examples include sugar, butter, and salt. Group 3 foods are made by adding components from group 2 to group 1 foods. These include canned beans, smoked meat, and cheese.

Group 4 foods are defined as industrial formulations made with several ingredients, such as salt, sugar, preservatives, stabilizers, and antioxidants. In addition, these foods often contain extracted components, such as gluten, protein isolates or concentrates, flavor enhancers, emulsifiers, and hydrogenated oils. Processing techniques include extrusion, pre-frying, and molding. Foods in this group include ready-to-eat products, pastries, sausages, artificially flavored yoghurts, and mass-produced breads. According to the developers of the NOVA system, foods belonging to group 4 should be avoided.[196,211,212]

2.2.4.2 Other food processing classification systems

Other classification systems use highly processed foods as the most processed category instead of the ultra-processed term as shown in **Figure 4**. As an example, a system which aims to classify foods based on the degree of industrial processing techniques has been developed by Poti et al.[199] as an alternative to the NOVA system. Their classification system also has four groups, including unprocessed and minimally processed, basic processed, moderately processed, and highly processed. Unprocessed and minimally processed group includes products that have gone through none or only very slight modifications, and processing techniques include drying, packaging, freezing, and removal of inedible parts. Basic processed group includes products that have gone through extraction, purification, canning, and fermentation. Moderately processed group includes foods from the first two categories that contain additives, such as sweeteners, flavors, or fats. Lastly, highly processed group is defined as industrially formulated mixtures of multiple ingredients, and the raw material is no longer recognizable in the product. However, this system has not been applied in epidemiological studies as extensively as the NOVA system.

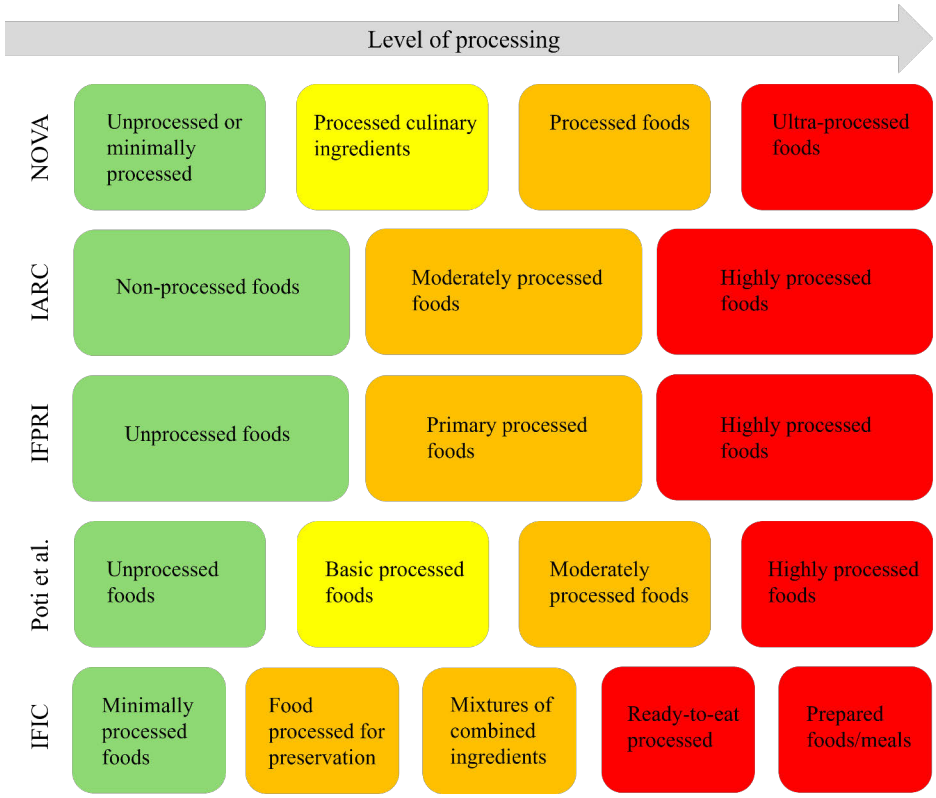


Figure 4. Group definitions for food classification systems NOVA, IARC, IFPRI. Poti et al. and IFIC. Green color indicates less processing while red color indicates more/harsher processing steps.

2.3 Metabolomics analysis

Metabolomics analyses can be used to identify various compounds in plant-based foods, the impact of processing techniques on their biochemical composition, to distinguish differences between animal-based products and their plant-based alternatives, quality control, authentication, and for the development of healthier plant-based foods overall. In addition, in food sciences metabolomics can be utilized to study dietary biomarkers, impact of environmental factors on plant growth, and sensory attributes of food products[213]. For industrial point of view, identification of metabolites associated with sensory properties can be useful when developing new products[214]. Metabolomics focuses on the analysis of small molecules present in the analysed material, which results in thousands of metabolites providing information regarding the comprehensive biochemical composition of the product. This wider approach to analyse the compositions of foods, instead of just focusing on some basic nutrients, has provided valuable

information of the 'dark matter' of food products[12,215]. Depending on the research question, targeted or untargeted metabolomics analysis can be used.

2.3.1 Metabolomics workflow in general

The basic workflow of metabolomics analysis consists of experimental design and sample collection, sample preparation and metabolite extraction, data acquisition, and finally data analysis[216]. Liquid chromatography coupled with mass spectrometry is often used for the analysis of plant-based foods. It is possible to analyse the semipolar plant secondary metabolites with it[217]. Their extraction is often done with aqueous alcohol solutions and with LC–MS, there is no need for derivatization before analysis, as GC–MS would require.[218] The analyses are often done in reversed phase and hydrophilic interaction chromatographies and both in positive and negative ionization modes. One reason for this along with the polarity of the compounds is, that for instance amino acids are ionized more efficiently in positive mode, whereas some oligosaccharides are in negative mode[219].

Data analysis includes peak picking from raw data and preprocessing it to ensure proper quality before statistical analyses and metabolite annotations. The annotation procedure for LC-, and GC-based metabolomics is based on matching the *m/z*, fragmentation patterns, and retention time to existing databases containing information analysed from standard compounds.[216,218] Metabolites can be identified with different ID levels depending on whether they were annotated based on chemical standards, existing spectral libraries, or based on physicochemical properties[220].

Software like MS-DIAL[221] can be used for the peak picking and data alignment, and R[222] with different packages *e.g.* notame[216] can be used for data preprocessing. With data preprocessing retention time drift can be corrected and low-quality features flagged and based on preferences removed from further statistical analyses[216]. In addition to statistical analyses, R[222] can be used to visualize metabolomics data efficiently. Principal component analyses, heatmaps, and other visual presentations make the data more interpretable as metabolomics data can often consist of tens to hundreds of thousands of metabolites.

2.3.2 Metabolomics analysis for plant-based protein-rich foods

In a study by Van Vliet et al.[27], where they compared grass-fed beef to plant-based alternatives using gas chromatography coupled with mass spectrometry (GC–MS) based metabolomics analysis, they found 171 differing metabolites between these products, even though the Nutrition Facts panels for the animal- and plant-based options were comparable. These results highlight the importance of the 'dark matter' of food products, which can be overlooked in other

commonly used analysis techniques. In a similar study by Hernandez et al.[223], they used metabolomics to compare plant-based meat alternatives to ground beef. They concluded that plant-based foods contained various bioactive compounds, while the ground beef products had higher abundances of long-chain fatty acids, peptides, and amino acids. In addition to the differences between animal and plant-based products, they were able to show differences between soy- and pea-based products. In another study by Fan et al.[224], they investigated the impact of adding soybean hull polysaccharides in plant-based yogurts using LC–MS. With a targeted metabolomics approach, they were able to determine 278 metabolites which were different between the plain yogurts and the ones with added soybean hull polysaccharides. Furthermore, they used pathway analysis to determine the formation and degradation of various metabolites. Lou et al.[225] used metabolomics to determine metabolites with sensory properties in soy-based foods. They found that during fermentation of soy-based meat alternatives, metabolites associated with meaty flavor and color, such as glutathione and β-carotene, were dependent on the used starter culture for the fermentation.

Therefore, metabolomics can be used to study PBPR foods for example by comparing them to their animal-based counterparts, by studying the impact of different processing techniques on them, or their health implications on humans.

3 AIMS OF THE STUDY

The aim of this thesis was to study the impact of various processing techniques, such as fermentation, on the biochemical composition of plant-based protein-rich foods using LC–MS metabolomics analysis. This thesis consisted of two studies.

The objectives of the individual studies were:

I. To study the impact of various processing techniques on the biochemical composition of commercially available plant-based foods. These foods were made with different raw materials and had different stages of processing varying from whole beans to products made with extracted components, such as protein concentrates. In addition, existing food processing classification systems were used to categorise these products.

II. To investigate the impact of different fermentation techniques on the biochemical composition of lupin-based beverages. Lupin beverages were fermented with two lactic acid bacteria strains, spontaneous, and kombucha fermentations.

4 MATERIALS AND METHODS

4.1 Plant-based foods

Commercially available plant-based foods and animal-based products used in study **I** were obtained from local stores in Turku area, Finland, from September to December 2021. The aim was to purchase majority of the commercially available plant-based foods made from various raw materials with varying processing techniques. In total, 168 plant-based food products were purchased representing different degrees of food technological processing, ranging from whole preserved beans to more processed products made with extracted plant-based components like protein concentrates. Unseasoned animal-based products, including beef, pork, fish, and chicken, were used as controls in the study. All of the food products were prepared based on the instructions on the packages to mimic the way they would be prepared at home by boiling or frying. Only exception was that frying oil was not used. For instance, dry extrudates were boiled and the animal-based foods were cooked. After preparation, all of the cooked and uncooked food products were stored at –20°C until further sample processing.

The plant-based foods were classified based on the raw material, product category, and the product type based on the protein type mentioned in the ingredient label. For the raw material, the first plant-based ingredient mentioned in the ingredient label was used since plant-based foods are often made from several plant-based ingredients, but the first one mentioned on the list is commonly the main ingredient. Based on raw materials foods were classified as chickpea, fava bean, oat, pea, soy, wheat, or other legume-based ones. The other legumes group included whole beans and products made from some raw materials, which were only individual products or did not have all product types available. These included black beans, white beans, kidney beans, red lentils, butter beans, green lentils, borlotti beans, and three products made from rice and sunflower seeds. Product categories were beans, extruded chunks, minced & pulled products, tofu, tempe, cold cuts, nuggets, balls, steaks & others, and sausages. For product type, these foods were divided into extruded chunks, whole bean/plant, tofu, tempe, and products made with protein concentrates or isolates based on the used protein type of plant-based raw material. Extruded chunks were made from whole bean/plant or from flour, and they had textural properties specific to extruded products. Tofu and tempe were easy to classify as they are usually mentioned in the packages. Whole bean/plant group included products such as whole preserved beans, or products which were made using to whole bean/plant as mentioned in the ingredient label. Last group were products made with protein concentrates or isolates which was mentioned in the ingredient

label, but the difference between concentrate or isolate was not always mentioned and therefore this group contained both of them.

4.2 Lupin beverage fermentations

In study **II**, the lupin flour (Sweet lupin flour) used in the fermentations was obtained from local farm Koivunalhon Luomutila (Lieto, Finland). This commercial flour was made from organically grown and peeled lupin seeds. Lupin-based beverages used in fermentations were prepared by mixing the flour with tap water in 1:10 (w/w) ratio and blending the mixture for 30 second at maximum speed (Chef XL Titanium with a blender attachment, Kenwood Limited, Havant, UK). After blending, the beverages were centrifuged (10 min, 5000 g, RT) to remove the excess solid materials. For the lactic acid bacteria fermentations, these beverages were stored at –20°C. For the spontaneous and kombucha fermentations these beverages were prepared freshly before the experiments. The single-strain LAB and spontaneous fermentations were made with a bioreactor in 1.5 L vessel containing 500 mL of the beverage (Minifors 2, Infors HT), while the kombucha fermentations were made in beakers in an incubator. Before the single-strain LAB fermentations, lupin beverages were autoclaved within the bioreactor to ensure the growth of only the inoculated bacteria. For the spontaneous and kombucha fermentations these beverages were not autoclaved. Each of the fermentations were done in triplicate. Samples were collected at various time points during each of the fermentations and stored at –20°C before further sample preparation.

4.2.1 Lactic acid bacteria fermentations

For the single-strain LAB fermentations, two individual strains (LAB 10492 and LAB 20174) were used from an in-house collection, and they had been originally obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ, Braunschweig, Germany). These strains had been stored at –80°C in MRS glycerol stocks. For this study, they were first preincubated for 24 h in MRS broth (acumedia Neogen, USA). After preincubation, the bacteria cells were washed twice by centrifuging (3500 rpm, 20 min, +8°C) and the remaining pellet was suspended in 0.9% saline. The viable cell counts for both strains were determined by plating dilution series on to MRS plates and incubating them at +30°C for 48 h.

After autoclaving the bioreactor with the beverage, it was let to cool down to room temperature before the fermentation experiments. The concentration of the inoculated bacteria was approximately 1×10^8 CFU/mL. Parameters for the fermentations conditions were +28°C, stirring of 100 rpm/min, and for 24 hours in total. Samples were collected throughout the fermentation, and pH was

monitored for the whole time with a sensor. Timepoints for sample collections were 0 h (after inoculation), 30 min, 1 h, 2 h, 4 h, 6 h, 8 h, and finally 24 h.

4.2.2 Spontaneous fermentation

The bioreactor was autoclaved without the lupin-beverage for spontaneous fermentations for sterilization. After cooling down the bioreactor, lupin beverage (500 mL) was added. Similarly with the single-strain LAB fermentations, parameters used were +28°C, stirring of 100 rpm/min, and pH was monitored with a sensor throughout the whole fermentation process. Spontaneous fermentations were continued until 96 h due to the slow decrease of the pH. However, it did not continue to decrease after 96 h during additional experiments, and therefore that was determined to be the end point of the fermentation.

4.2.3 Kombucha fermentation

The kombucha starter kit (Kombucha Starter Kit, Good Guys Oy) for kombucha fermentations was obtained from a local store. According to the instructions in the starter kit, the initial kombucha used for inoculations was prepared by boiling 2.7 L of tap water and adding 15 g of black tea (Forsman tea, Keisarin malja tee (Fairtrade black tea 87 %, pineapple pieces 6 %, orange pieces 3 %, natural flavor 2.5 %, lily 1.5 %), Aaro Forsman Oy) and letting it simmer for 20 minutes. Tea leaves were removed after 20 minutes, and 180 g of regular white sugar was added and dissolved. The temperature of the tea was let to cool down to 28–30°C before adding the SCOBY with the started liquid included in the starter kit. The kombucha was left at a dark place at RT for 11 days covered with a cheesecloth. For the other two kombucha fermentations, the kombucha starter was prepared in the same way, except that the previously prepared kombucha batch was used as the acidifying starter liquid for the new batch. For the fermentations, 5% v/v of the kombucha liquid was added to the lupin beverages. The fermentations were done in beakers covered with cheesecloths under static conditions inside an incubator. The incubator was set to +30°C, and the pH values were measured (LGG-pH meter 5) before sample collections. The end point for kombucha fermentations was determined to be 72 hours. By that time, pH had decreased to under 4 which was close to the final pH value in the single-strain LAB fermentations.

4.3 Sample preparation

4.3.1 Plant-based foods

In study **I**, the cooked and frozen plant-based foods (n=168) and animal-based products (n=8) were thawed before sample preparation. Pre-homogenization was done using food processor (Bamix model M133) at full speed (mode 2) for most of the products. Foods with harder texture, such as whole beans, were pre-homogenized by using a pestle and mortar. To ensure the efficiency of this pre-homogenization procedure, we performed three technical replicates from 15 food products with complex sample matrices. These replicates included products with visible spices, such as herbs. Otherwise, we performed one extraction from a sample. After pre-homogenization, approximately 200 mg of the product was weighed for the extraction, which was done with 80 % methanol (MeOH) in water. The amount of used extraction solvent was 400 µl per 100 mg of weighed sample. After adding the extraction solvent, the samples were vortexed at full speed (5s, RT) and 3 ceramic beads (2.8 mm; Precellys Ceramic kit) were added before the final homogenization (30 s^{-1}, 1 min × 2; Tissuelyser 2; Qiagen). With this extraction method, some compounds can still be retained in the disgarded protein fraction impacting their presence in the extract. Therefore, optimization of the extraction method would be important in future studies. Extraction was done by vortexing the homogenized samples (5s, RT) and leaving the samples at RT for 15 minutes. Remaining solid materials were removed by centrifugation (18,000*g*, 10 min, +4 °C). The remaining supernatants were collected (200 µL) and diluted with 80 % MeOH (800 µL) to 1:20 concentration and vortexed (5s, RT). Before the LC–MS analysis all samples were filtered with 0.2 µm PTFE filters into HPLC vials with inserts. In addition to the samples, quality controls were prepared by pooling 20 µL of each diluted sample and filtered in the same manner.

4.3.2 Lupin-based beverages

Frozen samples collected from the various time points were thawed before sample preparation. The sample preparation procedure was similar to the one used with plant-based foods, excluding the homogenizations which were not relevant with these lupin beverage samples. Extraction of the samples (200 µL) were done with 80 % MeOH in water by vortexing at full speed (5s, RT). After vortexing, samples were centrifuged (18 000g, 10min, +4°C) and the supernatants (200 µL) were diluted with 80 % MeOH in water (800 µL), vortexed with full speed (5s, RT), and filtered with 0.2 µM PTFE filters into the HPLC-vials with inserts. Similarly, 20 µL of each diluted sample was pooled and filtered for quality controls.

4.4 Metabolomics analysis

4.4.1 LC–MS analysis

The LC–MS analysis using ultra-high performance liquid chromatography combined with quadrupole time-of-flight mass spectrometry (LC-QTOF-MS) was used in studies **I** and **II**. In both studies, samples were analysed using reversed-phase (RP) and hydrophilic interaction (HILIC) chromatographies. In study **I**, HILIC analysis was done with Elute UHPLC 1300 coupled with Bruker Impact II QTOF instrument from Bruker Daltonics. The RP analysis in study **I**, and both HILIC- and RP-analyses in study **II** were analysed with Agilent 1290 Infinity II UPLC coupled with 6546 LC-QTOF. Columns used in this study were Zorbax Eclipse XDB-C18, 1.8 µm, 2.1 mm × 100 mm; Agilent Technologies for RP, and Acquity UPLC BEH Amide, 1.7 µm, 2.1 mm × 100 mm; Waters Corporation for HILIC.

For HILIC, mobile phases consisted of 1:1 acetonitrile in water (solution A) and 9:1 acetonitrile in water (solution B), both containing 20 mM ammonium formate (Sigma-Aldrich). The gradient was 0–2.5 min, 100% B; 2.5– 10 min, 100% B to 0% B; 10–10.01 min, 0% B to 100% B; 10.01–12.5 min, 100% B with 0.6 ml min−1 flow rate. For RP, mobile phases were water (solution A) and methanol (solution B), with both containing 0.1% v/v formic acid. The gradient used was 0–10 min, 2–100% B; 10–14.5 min, 100% B; 14.5–14.51 min, 100–2% B; 14.51–16.5 min, 2% B with 0.4 ml min−1 flow rate. For both chromatographies the injection volume was 2 µl and sample tray was kept at +4 °C, and electrospray ionization was used with positive and negative modes. The following source parameters were used: drying gas flow, 10 l min−1; temperature, 325 °C; sheath gas flow, 11 l min−1; temperature, 350 °C; nebulizer pressure, 45 psi; capillary voltage, 3,500 V; and nozzle voltage, 1,000 V. In the full scan mode, the scan range was set to 50–1,600 m/z, scan time to 1.67 Hz and abundance threshold to 150. Quality controls were injected at the beginning of the analysis to prime the system and after every 12 samples to monitor the stability of the LC–MS analysis. Samples were analysed in a randomized order. Collision energies of 10 eV, 20 eV and 40 eV were applied in the separate MS/MS scans for the quality control and selected samples. Parameters for MS/MS scans were an abundance threshold of 200, target of 25,000 counts per spectrum, scan rate of 3.33 Hz, scan range of 50–1,600 m/z, maximum of 4 precursors per cycle, precursor isolation width of 1.3 Da, active exclusion after 2 spectra and release after 0.25 min. The data acquisition software used was MassHunter Workstation Acquisition 11.0 (Agilent Technologies).

4.4.2 Data analysis and metabolite annotations

In both studies, MS-DIAL[221] (v.4.80) was used for automated peak picking and alignment of the raw data. Parameters used in study **I** were 0.005 Da for MS tolerance, 0.015 Da for MS/MS tolerance, m/z range of 0–2,000 Da, a minimum peak amplitude of 6,000 signal counts, a mass slice width of 0.1 Da. In study **II**, parameters used were 0.01 Da for MS tolerance, 0.025 Da for MS/MS tolerance, m/z range of 0–2,000 Da, minimum peak amplitude of 2,000 signal counts, and mass slice width of 0.1 Da. In both studies smoothing level of 3 scans and minimum peak width of 5 scans were used. For peak alignment in study **I** and **II** retention time tolerance of 0.2 min and MS1 tolerance 0.015 Da were used, as well as gap filling by compulsion. In positive mode the adduct ions were [M + H]+, [M + NH4]+, [M + Na]+, [M + CH3OH + H]+, [M + K]+, [M + ACN+H]+, [M+H-H2O]+, [M+H-2H2O]+, [2 M + H]+, [M-NH4+H]+, and for negative mode [M − H]−, [M − H2O − H]−, [M + Cl]−, [2 M − H]−, [M+HCOOH-H]−. After the data alignment, results from both RP and HILIC and negative and positive ionization modes were exported to excel. In study **I**, this was 100,371 molecular features in total, and in study **II**, 52,316 molecular features. The raw data was then processed with notame package for data drift correction and flagging low-quality features[226] using the quality control samples.

In study **I**, from the 100,371 molecular features the most abundant 8,000 features based on the maximum average peak areas for each plant-based materials with the remaining 1,286 features with MS/MS data were selected for further analyses. In study **II**, from the 52,316 molecular features 35,997 features were selected for further analyses by calculating the maximum value of sample abundance to blank ratio to remove features from possible contaminations. Metabolite annotations in both studies were done using in-house databases and publicly available spectral databases (all of the ESI MS/MS databases available at the MS-DIAL database repository, including *e.g.* MassBank, ReSpect, GNPS, Fiehn lab and RIKEN databases) by comparing *m/z*, retention time and MS/MS fragmentation patterns. ID level 1 refers to compounds identified based on chemical standards, level 2 to putatively annotated compounds matched with spectral libraried, level 3 to putatively annotated compound classes and level 4 to unknown compounds[220].

4.4.3 Statistical analysis

As both studies were exploratory and consisted of various different sample types, employing statistical analyses proved to be challenging. Commonly used statistical methods *e.g.* ANOVA did not provide additional insights, as the differences between sample types/groups were already evident from multivariate analyses. Principal component analysis (PCA) for all plant-based products and

animal-based ones in study **I** and for all fermented beverages in study **II** were prepared with *z*-normalized and log transformed data using *ggbiplot* package with R. For soy-based products in study **I**, PCA with Pareto scaling, mean centering, and log transformations were prepared with SIMCA v.16 (Sartorius Stedim Data Analytics). *k*-means clustering analyses for study **I** and **II** were prepared with *z*-normalized data in R with *ComplexHeatmap*. In study **II**, lineplots for annotated compounds were prepared with *ggpubr*.

5 RESULTS AND DISCUSSION

5.1 Impact of processing on the biochemical composition of plant-based foods

In study **I**, the impact of processing on the biochemical composition of plant-based foods was examined using non-targeted metabolomics approach. The commercial products (n = 168) analysed in the study were made with various raw materials and with varying processing techniques. In addition, the biochemical composition of unseasoned animal-based products (n = 8) was analysed and compared to plant-based foods.

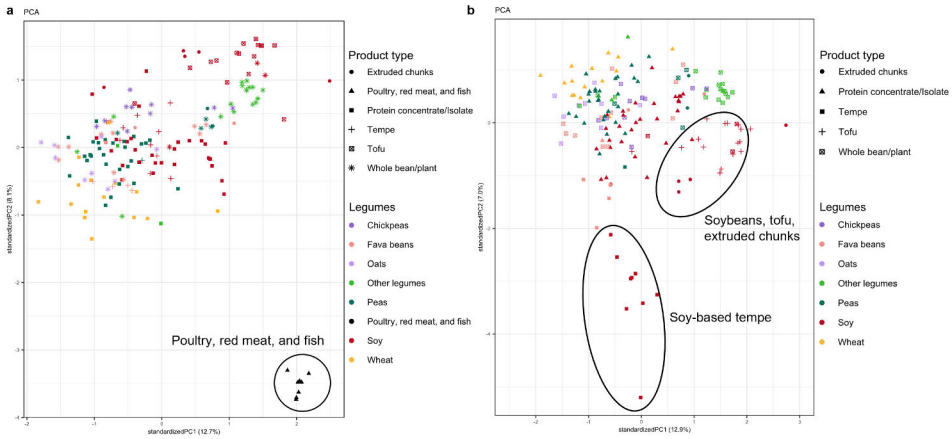


Figure 5. Principal component analyses of plant- and animal-based foods made with good-quality molecular features (n = 9389) obtained from non-targeted LC–MS based metabolomics analysis. **a.** Principal component analysis comparing PBPR foods made from various raw materials to animal-based foods (n = 176). **b.** Differences in the biochemical compositions of plant-based foods (n = 168). Principal components 1 and 2 (PC1 and PC2) and the variances by both are indicated by the percentages. Figure reprinted from Raita, et al. (2025)[53].

As expected, the biochemical composition of plant-based foods made from various raw materials was notably different compared to the animal-based products. In the PCA (**Figure 5a**) made with good-quality molecular features (n = 9389), all of the animal-based products clustered on the right bottom corner of the plot, while the commercial plant-based foods were more spread out on the upper side of the plot. Animal-based products seemed to have similar compositions even though they were derived from different animals. Compounds found to distinguish plant-based products from animal-based ones include for instance flavonoids, phenolic acids, and long-chain fatty-acids.[223] Therefore, the difference in the presence of these compounds in the food products is probably the reason for their separation. Animal-based foods can also contain

phytochemicals depending on the diet of the animal, for instance grass-fed beef can contain higher quantities of some phytochemicals, such as hippuric acid, succinic acid, and 4-hydroxybenzoic acid, compared to grain-fed beef[227]. Succinic and 4-hydroxybenzoic acids were also detected in animal-based samples in our study.

When further focusing on the plant-based foods, the impact of raw material was also noticeable as certain products separated from others (**Figure 5b**). Foods made from plant-based sources such as chickpeas, oats, and peas were found to have more similar biochemical compositions, while products made with soy separated from them. The reason why most of the other plant-based products appeared to have similar biochemical compositions might be that they were prepared using several plant-based ingredients to improve the nutritional quality of the product, for instance to improve amino acid composition. It is also clear that plant-based raw materials have different biochemical compositions as previously observed with soy- and pea-based products[223], but the impact of other added components can also result in these differences. Different seasoning and ingredients added to improve physicochemical properties can be behind this phenomenon.

5.1.1 Soy-based products

Soy-based products (n = 62) made with varying techniques were used for further analyses to examine the impact of processing on their biochemical composition. This group included products from different processing stages, from raw beans to mildly processed, and to products made with refined components like soy protein isolates. Such diversely processed products were not found within the ones made with other plant-based sources. These products were categorised based on the product type into whole bean products, extruded chunks, tempe, tofu, and products made with protein concentrates or isolates. Whole soybeans are used in the preparation of tofu and tempe, while soy flour is used in protein extraction and often in extrusion processes. For tempe fermentation, whole soybeans are used by inoculating the starter culture, and with tofu the whole beans are first cooked and grinded, followed by separation of the solid pulp and liquid part which is then further used in the final preparation of tofu. Against common misconception, tofu is not a fermented product. However, it is worth noting that the exact processing techniques used in the preparation of these products are not clear, as the producers rarely mention those in the packages. In case of protein concentrate and isolate products, it would be interesting to know the exact extraction method so it could be taken into account in the analyses. Same applies to extruded products.

When examining the biochemical composition of soy-based products with PCA using annotated compounds (n = 193), the main reason behind their separation in the PCA was the difference in their product types (**Figure 6a**). Whole bean products seemed to have similar composition with tofu, while fermented foods exhibited similarities with extruded chunks. However, products made with protein concentrates or isolates separated from these other products. The reason for these more processed products to separate might be due to added ingredients, such as spices and herbs. These kinds of added ingredients are known to provide bioactive compounds into the product, but the main contributor for the presence of bioactive compounds in the product is the used plant-based raw material itself. In addition to added ingredients providing bioactive compounds, these more refined products can contain more added fat and other additives.

As shown with the k-means clustering analysis in **Figure 6e**, products made with protein concentrates or isolates contain flavonoids most likely due to these added spices, as otherwise these compounds are often lost during protein extraction. In addition, this analysis clearly differentiates these product types based on their biochemical compositions, as compounds present in cluster 3 are found in tempe products, compounds present in cluster 2 in products made with protein concentrates or isolates, in cluster 1 in extruded chunks, cluster 4 mainly in tofu, and in cluster 5 compounds found in tempe, extruded chunks, and beans. Fermentation has been known to improve the bioavailability of amino acids[228], and these compounds are highly abundant in cluster 3 in tempe products.

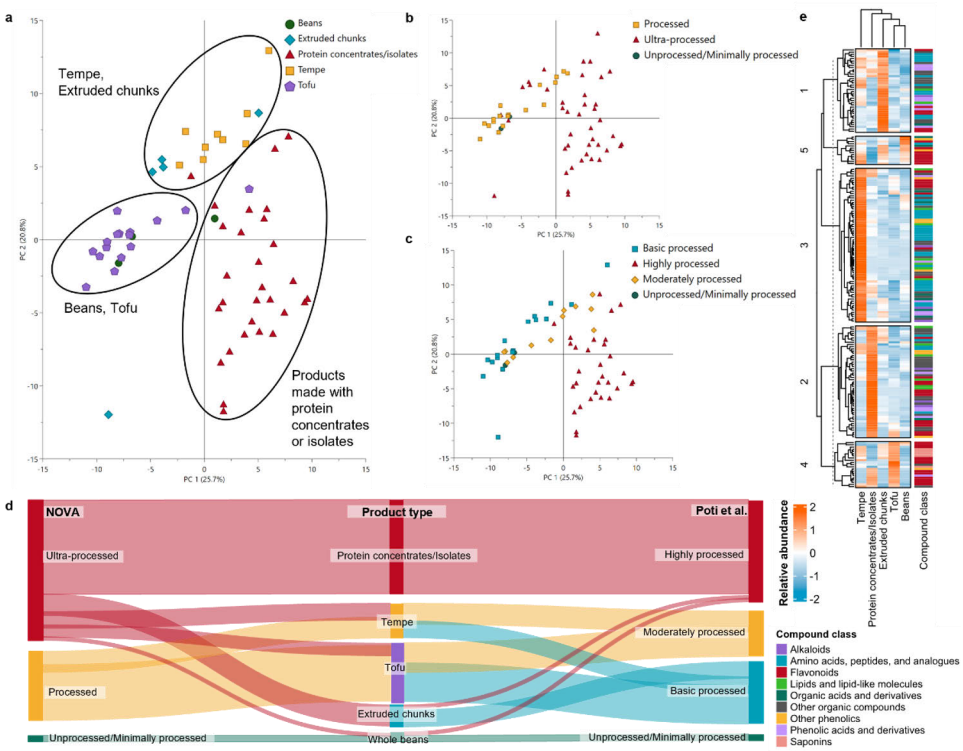


Figure 6. Impact of processing on soy-based products made with various processing techniques. **a**. Principal component analysis made with annotated compounds (n=193) for soy-based products classified by product types. **b**. Soy-based products classified based on the NOVA classification system in the same PCA model. **c**. Soy-based products classified based on the Poti et al. classification system in the same PCA model. **d**. Sankey diagram demonstrating the classification of soy-based products based on product type, NOVA, and Poti et al. systems. **e**. k-means clustering analysis made with the annotated compounds (n=193) in soy-based products. Data has been *z*-normalized. Figure reprinted from Raita, et al. (2025)[53].

In addition to the product types, we used existing food processing classification systems NOVA[196] and system developed by Poti, et al.[199] to categorise these products. When NOVA system was applied in the PCA, ultra-processed foods were found next to processed and minimally processed products (**Figure 6b**). The reason for this is that NOVA classifications are mainly made based on the ingredient lists (number of added components and their types) rather than based on the impact of processing on the biochemical composition of the product. Because of this, the definition between processed and ultra-processed or minimally processed and ultra-processed is sometimes misleading and can be

confusing. Most of the plant-based foods included in this study fall into the ultra-processed category, as they have added ingredients or components that are considered to be found only in ultra-processed foods. For instance, these include extracted components, such as protein concentrates or isolates. When a classification system developed by Poti et al. was applied (**Figure 6c**), separation of differently classified products was slightly better than with the NOVA system, but highly processed products were still found next to basic or moderately processed ones. With this system, the classification of products seemed to be more justified, but there is still room for improvement.

The sankey diagram (**Figure 6d**) demonstrates the difference between these classification systems, as the classification of foods based on product types is visually presented. Based on NOVA system, few of the tempe and tofu products are ultra-processed but based on the Poti et al. system they are classified as moderately processed. The reason for these products to be classified as ultra-processed is that they have been industrially pre-fried and/or contain some added ingredients, which should only be found in ultra-processed foods based on NOVA[212]. The whole bean product falling into ultra-processed and highly processed categories is a burger steak, which has been made from whole soybeans according to the ingredient label, but still contains several added ingredients impacting its classification level. These results demonstrate the diversity of plant-based foods belonging to the ultra-processed category. Even if some plant-based products classified as ultra-processed have been found to contain less micronutrients or protein when compared to animal-based ones[229], the ultra-processed label does not instantly indicate that the product has lower nutritional quality than for instance a processed product has, as their biochemical compositions are not taken into account in NOVA. In case of industrial frying, it does not automatically result in worse outcomes than home cooking, or is home cooking always a better option regarding harmful compounds. Home cooked foods have been found to contain comparable levels of harmful compounds, including acrylamide and compounds derived from Maillard reaction[230]. Nutritional profile of the product should be the main aspect when defining its healthiness.

While the development of these classification systems is important in order to educate people on healthier dietary choices, these systems should be further developed to consider more comprehensively the biochemical composition of food products and the impact of different processing techniques on them. Currently, they do not consider what is taken away from the raw material or the formation of new compounds due to processing techniques. Plant-based products are often categorised as ultra-processed, because they can contain various added ingredients or additives. Even if they have been processed with beneficial techniques, such as fermentation, they can still be misleadingly classified as

ultra-processed[53]. While there is clear evidence that majority of ultra-processed foods have adverse health effects, plant-based meat alternative foods have not been associated with these effects like some highly processed animal-based products and sugary beverages have[207,231].

5.1.1.1 Isoflavonoids in soy-based products

One specific group of compounds in soy-based products that were affected by different processing techniques were isoflavonoids. These compounds are also known as phytoestrogens, which can have various health effects on humans. In addition to the impact of processing on isoflavonoids, maturity of the beans and their water content have major impacts on their quantities[232].

The abundances of isoflavonoids in variously processed soy-based products are expressed in **Figure 7**. In **Figure 7a**, products are shown with sample codes which are based on their product types (Te, tempe; E, extruded chunks; T, tofu; C, protein concentrates/isolates; B, beans). Products which were made with protein concentrates or isolates seemed to have lower abundances of these compounds indicating, that their relative quantities are most likely diminished during the protein extraction process, as the fiber fraction likely containing them is being removed. Foods made from protein concentrates or isolates are often consumed together with other foods, which may help balance the intake of phytochemicals. In addition, processing techniques can have an impact on the form of these compounds, as the abundances of acetyl derivatives were highest in extruded products. This has also been observed in previous studies as high temperatures can increase these specific derivatives[137,232]. The aglycone forms were mainly found in fermented tempe samples, while malonyl derivatives were found in whole bean products, tofu, and extruded chunks. Malonyl derivatives are often found in whole soybeans, as they are the stable storage forms of these compounds[233].

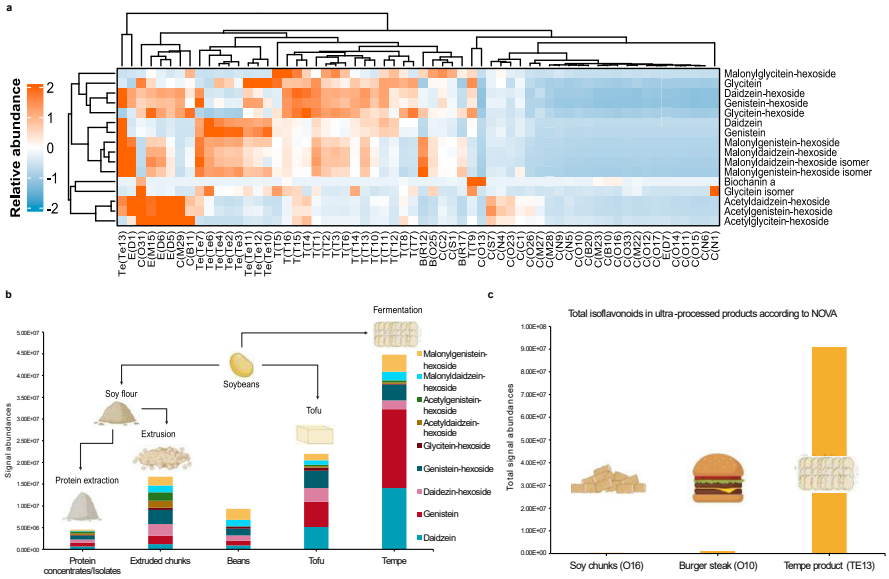


Figure 7. Isoflavonoid abundances in soy-based products. **a.** Abundances of isoflavonoids in soy-based products expressed in a heatmap. Sample codes are shown in parentheses. **b**. Abundances of isoflavonoids in different soy-based products. **c**. Visualization of total isoflavonoid abundances in three different soy-based products categorised as ultra-processed. Figure reprinted from Raita, et al. (2025)[53].

From **Figure 7b** it is obvious that fermented products have highest abundances of aglycone forms. Similarly in other studies, concentrations of the aglycone forms of isoflavonoids have increased after germination[234]. These forms have the highest bioavailability for humans, as their absorption is faster than their glycoside forms[154]. Normally isoflavonoid glycosides are hydrolyzed to their aglycone forms in the small and large intestines, but during fermentation or germination the microorganisms can already break the glycoside bond[72]. However, our study focuses on relative abundances rather than quantification, and therefore conclusions regarding the bioavailability of isoflavonoids in these products cannot be drawn. In addition to the aglycones, fermented products with extruded chunks and tofu seem to have higher total abundances of isoflavonoids compared to beans and products made with protein concentrates or isolates. The low abundance of isoflavonoids in beans can result from inefficient extraction as they might be bound into the fibers present in beans. Isoflavonoids can also be lost due to leaching[235], but the content of aglycones has been found to increase

during preparation of tofu as the glycoside bonds are probably hydrolyzed during soaking and by addition of heat[236].

In **Figure 7c**, three different products classified as ultra-processed and their total isoflavonoid signal abundances are shown. These abundance values indicate the presence of these compounds in the products. In the extruded soy chunk and burger steak isoflavonoids are relatively absent, while that one tempe product has a high abundance of them. This specific tempe product was classified as ultra-processed because it contained acidifying agent, which can be used to enhance flavors in products. Whether the addition of this ingredient makes the product to be avoided as recommended within the NOVA system, remains debatable.

5.2 Biochemical composition of lupin-based beverages

In the second study, the focus was on the impact of different fermentation techniques on the biochemical composition of lupin-based beverages. We used two lactic acid bacteria strains, spontaneous, and kombucha fermentations and analysed the samples using the same LC–MS metabolomics approach as was used in study **I**. We aimed to compare the impact of specified fermenting bacteria to unknown microorganisms, and how the biochemical composition of the lupin beverage differs between these fermentation techniques. Lupin was chosen as the fermentable raw material, as it has high content of protein and fiber, and could be utilized when producing more environmentally sustainable plant-based options in the future.

5.2.1 Impact of fermentation on various compound classes

The impact of different fermentation techniques on the biochemical composition of lupin-based beverages was evident, as the LAB fermentations resulted in different composition than spontaneous and kombucha fermentations. These LAB fermentations consisted of single-strain bacteria, while kombucha and spontaneous ones consisted of multiple microbial species. However, the microbial compositions of kombucha and spontaneous fermentations were not determined in this study. Both of the single-strain LAB fermentations exhibited similarities, while spontaneous and kombucha fermentations exhibited similarities among each other (**Figure 8**).

The pH values were monitored to follow the succession of each fermentation. The end points for fermentations were chosen based on the pH values, and for the single-strain LAB fermentations these were based on preliminary tests and existing literature[163]. For spontaneous and kombucha, we aimed for similar pH values to be used as the end points. Additionally, longer fermentation time was

tested for spontaneous fermentation, but the pH did not continue to decrease. Kombuchas generally have pH of 2.5–3.5 and for these fermentations the end values ranged from 3.5 to 3.9.

One significant reason behind the differences in the composition between these beverages might be in the preparation. For the single-strain LAB fermentations, lupin beverages were autoclaved prior inoculation to ensure sterile conditions. However, autoclaving would result in the loss of the microorganisms present in the material that are vital for the succession of spontaneous fermentations, and thereby it was not used for those ones. For kombucha fermentations, lupin beverages could have been heat treated before and this step would probably be necessary if they would be intended for consumption. In **Figure 8a**, different time points in the single-strain LAB fermentations appear to be closer to each other than in spontaneous and kombucha. This observation indicates that the biochemical composition of these beverages changes more drastically during spontaneous and kombucha fermentations, probably due to the differences in their microbial compositions. Already at the 24 hour time points separation can be seen in spontaneous and kombucha, but most of the changes appear to happen after that. Perhaps if the fermentation would have been continued for both single-strain LAB fermentations, similar effects would have been seen. Overall, the impact of heat treatment can be seen between these fermentations, and it has been previously shown to decrease the levels of bioactive compounds in lupin flours[237]. Because of these differences in the pH values, fermentation times and pre-treatments, these beverages are not biologically comparable. Still, the impact of these different fermentation techniques is visible and provides novel information.

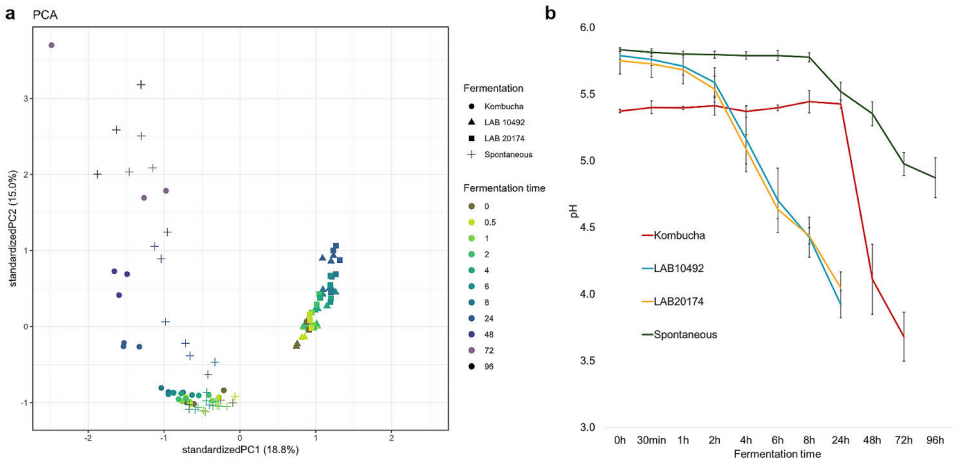


Figure 8. Biochemical composition of differently fermented lupin-based beverages. **a**. Principal component analysis made with good-quality molecular

features (n=35,996) for kombucha (n=30), LAB 10492 (n=24), LAB 20174 (n=24), and spontaneous (n=33) fermentations. Time points are expressed in different colors. **b.** pH values for each fermentation technique with standard deviations from the three replicate fermentations.

Similarities and differences between these fermentation techniques are visible in the k-means clustering analysis (**Figure 9**) made with the annotated compounds (n = 155). Clusters 3 and 2 show similar behaviors, as compounds present in the beginning of the fermentations decrease towards the end in cluster 3 and vice versa increase in cluster 2. Moreover, clusters 4, 5, and 6 demonstrate the different behaviors, as single-strain LAB fermentations behave similarly likewise do kombucha and spontaneous fermentations. In cluster 1, LAB 20174 fermentation differentiates from LAB 10492 regarding some of the compounds. These compounds include for instance aconitic acid and quinolizidine alkaloids, like lupinine and oxymatrine. For some reason their abundances are lower already from the start point of the fermentation in LAB 20174. Most likely this results from some difference during the autoclaving process, as otherwise these beverages were prepared in the same way.

Amino acids are a group of compounds that could be affected by the autoclaving procedure of the beverages. High temperatures result in protein denaturation and can decrease the quantities of amino acids in the product. Autoclaving used as sterilization procedure has been shown to result in lower total protein content and lower concentrations of amino acids[238,239]. However, in a study by Pontes et al.[237], they discovered that heating increased the content of protein in lupin flours. In addition, they stated that while the actual amount of protein in the product does not increase due to processing, its digestibility and bioavailability can. The abundances of amino acids, such as leucine, proline, and isoleucine, are low throughout the fermentation when done with both of the LAB strains but increase at the end in kombucha and spontaneous. Moreover, their abundances increase earlier in the kombucha fermentation than in spontaneous. As expected, the abundance of sugars, such as sucrose, trehalose, and melibiose, decreases towards the end as they are utilized by the bacteria. Previously, LAB have been shown to decrease the content of sugar by up to 20 % after fermentation[240].

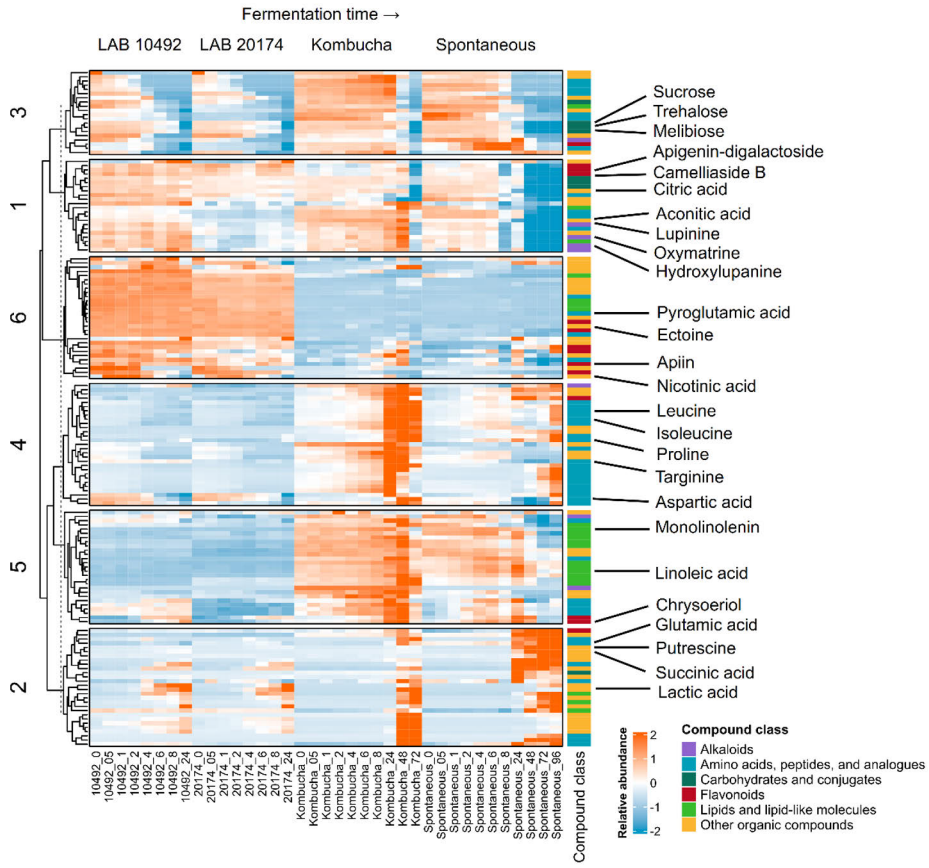


Figure 9. k-means clustering analysis of annotated compounds (n=155). Results of a k-means clustering analysis expressed in a heatmap made with *z*-normalized data using average signal abundances obtained from the three fermentation replicates for each technique. Compound classes for annotated compounds are expressed in different colors.

5.2.2 Changes in the biochemical compositions of lupin beverages

One class of compounds affected by fermentation were raffinose family oligosaccharides. Raffinose and stachyose were within the annotated compounds, and the changes in their relative abundances are shown in **Figure 10**. In the single-strain LAB fermentations their abundances stay rather similar during the whole fermentation process, while after 24 hours their abundances decrease almost completely in spontaneous and kombucha fermentations. Perhaps such effects could have been seen in the single-strain LAB fermentations if they had been continued for longer than 24 hours. Additionally, use of other LAB strains might have resulted in different outcomes. However, this could also be due to the presence of other microorganisms. In other studies, fermentation has been shown to decrease their content[241]. These compounds are often considered to be

unwanted, as they are not digested in the small intestine and can therefore cause flatulence[242]. However, they can also have beneficial effects, as they can act as prebiotics[243].

The formation of biogenic amines seems to be related to the fermentation type and culture (**Figure 10**). During fermentation, these compounds are formed from free amino acids and their formation is often associated with fermentations without a starter culture[244,245]. Kombucha fermentation increases the abundances of phenylethylamine, tyramine, and spermidine, while putrescine is mainly increased in spontaneous fermentation. Both of the single-strain LAB fermentations did not increase the abundances of these compounds, except for spermidine which started to increase towards the end point. Tyramine and putrescine are found to be the most dominant biogenic amines in fermented plant-based foods, but interestingly these and other related compounds were not detected from kombucha in another study[246]. However, spontaneously fermented vegetables have been associated with high levels of biogenic amines, possibly impacting their healthiness[247].

Lactic acid and its derivatives indolelactic acid (ILA) and phenyl lactic acid (PLA) are produced by LAB. Their abundances increase earlier in both of the single-strain LAB fermentations than in spontaneous and kombucha. The reason for this could be that the presence of LAB in spontaneous and kombucha is probably lower than in the LAB inoculated fermentations, and they could even be different species. The content of these compounds has been found to increase in LAB fermentations in other studies[248]. ILA is produced from tryptophan[249], while PLA can be produced from phenylalanine and tyrosine[250]. Lactic acid and its derivatives have beneficial properties, such as improving food preservation and safety[251,252].

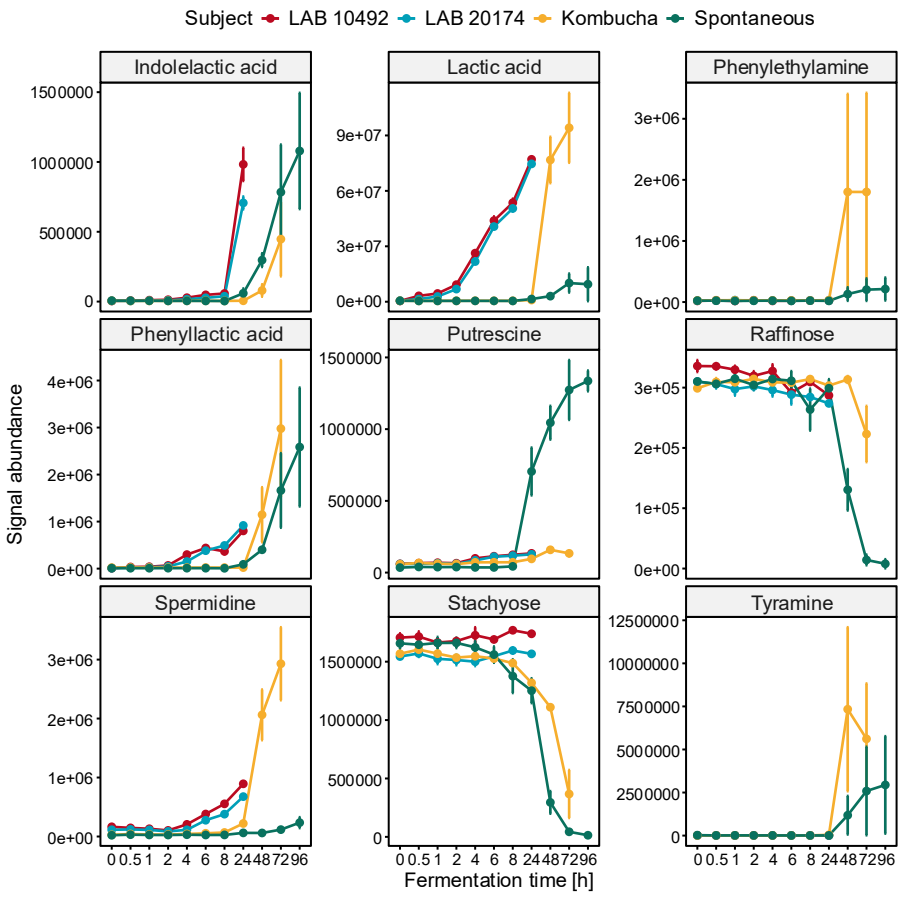


Figure 10. Relative signal abundances of indolelactic acid, lactic acid, phenylethylamine, phenyllactic acid, putrescine, raffinose, spermidine, stachyose, and tyramine in each of the fermentation techniques. Standard deviations for the three replicate fermentations are expressed as vertical lines.

Vitamin B compounds (**Figure 11**) are important for basic human health. This group of vitamins consists of eight compounds, and they can be obtained from various nutrition sources. Vitamin B3 compounds nicotinic acid and niacinamide are important for their antioxidative properties and energy production. If the dietary intake of these vitamins is low, it can result in a skin condition called pellagra.[253,254] Vitamin B1 has been associated with energy production and carbohydrate metabolism, and inadequate intake from diet can lead to several symptoms, such as brain fog, mood swings, and fatigue[255].

In these beverages, the impact of autoclaving can be seen with niacinamide, vitamin B3 derivative. Its abundance is already higher in the single-strain LAB fermentations at 0 time point indicating that heat treatment might increase its content. However, as its content decreases during fermentations the abundance of nicotinic acid (vitamin B3) simultaneously increases. In the single-strain LAB

fermentations this change happens during the first hours, while in spontaneous and kombucha fermentations it happens only after 24 hours of fermentation. LAB can form nicotinic acid from niacinamide through deamidation process[256], which can explain this observation. Similar behavior has been observed with fermented fruit juices, as the content of nicotinic acid increased after fermentation with LAB[257]. However, the sudden decrease of nicotinic acid after first observed increase might be explained by it's use as a growth factor by lactic acid bacteria[258]. The changes in the abundances of niacinamide and nicotinic acid demonstrate faster fermentation process in the single-strain LAB fermentations, as they probably had higher content of bacteria present.

While the abundances and changes of niacinamide and nicotinic acid during fermentation might be related, the abundance of thiamine (vitamin B1) decreases in each of the fermentations. In both of the LAB fermentations, its abundance in the beginning of the fermentation is slightly lower than in spontaneous and kombucha. In cow's milk, heating has not been found to have an effect on the quantity of thiamine[259]. In tea-based kombucha fermentations, the content of vitamin B1 has been found to increase[260], which was not observed here. Overall, the decrease of the abundances of these compounds is unwanted, as they can have several health beneficial effects. In order to retain these compounds, shorter fermentation times could be employed. In addition, testing and selecting fermenting bacteria which produce these compounds would be useful.

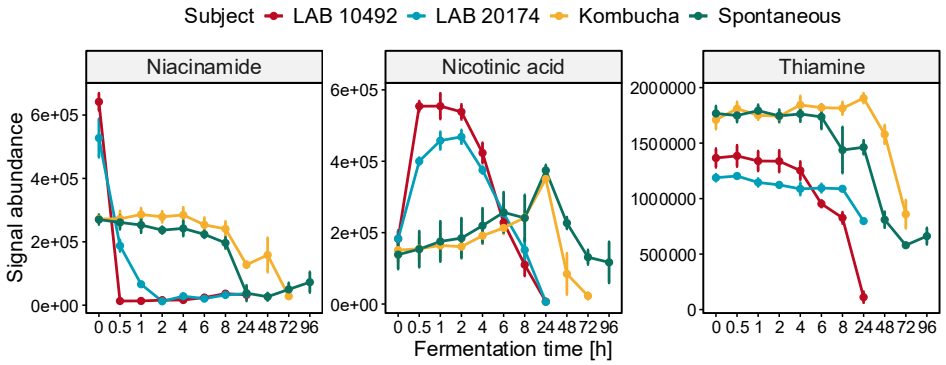


Figure 11. Relative signal abundances of niacinamide, nicotinic acid, and thiamine in each fermentation. Standard deviations for the three replicate fermentations are expressed as vertical lines.

5.3 Strengths and limitations of the studies

Both studies **I** and **II** have strengths but also some limitations. Metabolomics analysis has strengths as a wide range of metabolites were analysed in both

studies. By using RP and HILIC chromatographies in both positive and negative ionization, metabolites with different chemical properties can be analysed.

Study **I** provides valuable information of the impact of various processing techniques on the biochemical composition of PBPR foods. This study consisted of a large set of commercial plant-based foods made with varying raw materials and processing techniques. As comprehensive analysis of the metabolite composition of PBPR foods has not been conducted. With soy-based products we were able to demonstrate the impact of processing on bioactive compounds, and additionally the inefficiency of existing food classification systems. For study **II**, strengths include the novelty of the study as it provides insight into the application of lupins for the production of plant-based alternatives in the future. We showed that fermentation can have beneficial effects, as the abundances of lactic acid derivatives with beneficial health impacts increased, while the abundances of RFO's associated with negative outcomes decreased. Additionally, this study included novel production of lupin-based kombucha beverage.

The results obtained in study **I** are significant for the development of food processing classification systems in the future. It highlights the importance to consider the comprehensive biochemical composition of PBPR foods in addition to their nutritional content, instead of focusing solely on the used processing technique or basic nutrients. The presence of phytochemicals in the PBPR foods indicates, that their abundances were dependent on the used processing technique. Fermentation seemed to increase their abundances, while protein extraction resulted in lower abundances. They are important compounds to consider, as they can be related to the health benefits associated with plant-based foods. Both of the studies emphasize fermentation as a beneficial processing technique which could be used more in the future for the production of healthy plant-based foods. The results from study **II** could be employed when developing beverages or other food products from lupins. This underutilized raw material could provide protein-rich sustainably produced options to animal-based products as it can also be cultivated in northern latitudes.

However, there are some limitations in the studies. Limitations within the metabolomics analysis include that we use relative abundances instead of exact quantities. Therefore, the observations related to the related abundances of compounds and thereby their possible impacts on health are only figurative. High abundance does not necessarily reflect high bioavailability of a compound, as it is a combination of various factors, such as the food matrix, individual factors, and gut microbiota. In addition, a great number of metabolites remain as unknows due to inefficient fragmentation or the lack of comparable standard compounds in our in-house database and publicly available databases. Within the sample analysis, some compounds can be bound to the fiber or protein matrix

which can result in their inefficient extraction. The use of a digestion model or a clinical intervention trial would have provided valuable information to support the obtained findings in relation to their possible health effects, but these approaches were outside the scope of this thesis. However, such experiments have been conducted with the same products used here.

In study **I**, additional limitations include the complexity of the biochemical composition of PBPR foods which can be challenging when identifying phytochemicals. The study design in study **II** regarding the duration of fermentations and their pH at the end could have been determined more carefully for more reliable comparison between the fermentations. In addition, the impact of different pretreatments on the fermentations provides additional challenges. Sensory analysis and determination of the final microbial compositions would have provided valuable information for the study.

6 SUMMARY AND CONCLUSION

In this thesis, non-targeted metabolomics approach using liquid chromatography coupled with mass spectrometry was used to study the impact of various processing techniques on the biochemical composition of plant-based foods. The aim was to determine how these different techniques, such as fermentation, can modify the composition of plant-based products. In study **I**, commercial plant-based products made from different raw materials and with varying processing techniques were analysed. These products consisted of various product types, such as whole beans, extruded chunks, tempe, and tofu. In addition to their product types, existing food processing classification systems were used to categorise these products. Study **II** consisted of fermentation of lupin-based beverages and their analysis to see how fermentation can modify their biochemical composition. Employed fermentation techniques included lactic acid bacteria fermentation using two different bacteria strains, kombucha fermentation using commercially obtained SCOBY, and finally spontaneous fermentation with microorganisms present in the raw material.

In study **I**, the impact of processing on the biochemical composition of protein rich plant-based foods was evident with the abundances of phytochemicals. Along with the plant-based foods, animal-based foods were analysed and their compositions differed drastically from PBPR foods. Additionally, the impact of raw material on PBPR foods was shown and the analyses were further continued with soy-based products. Fermentation stood out as a technique that increased the abundances of isoflavonoid aglycones, while protein extraction seemed to decrease their abundances in products made with protein concentrates or isolates. Furthermore, when existing processing classifications were used to categorise PBPR foods, some conflicting results were obtained as products with high abundances of isoflavonoids, indicating the preservation of healthy whole-plant parts, were categorised as ultra-processed.

In study **II**, the aim was to investigate the impact of different fermentation techniques on the biochemical composition of lupin-based beverages. Two lactic acid bacteria strains were used and compared with each other, with addition to spontaneous and kombucha fermentations which are based on several microorganisms. The two single-strain LAB fermentations showed similar results and likewise did spontaneous and kombucha with each other. The difference between LAB fermentations and spontaneous and kombucha were probably due to the different bacteria, as well as heat treatment employed before LAB fermentations. Additionally, different fermentation times and final pH values might have resulted in the differences.

Food processing is often necessary for plant-based foods, but existing food processing classification systems can result in confusing categorisations. PBPR foods can be categorised as so-called ultra-processed foods even if they have been prepared with beneficial techniques, like fermentation. This study demonstrated that metabolomics can be used to assess the impact of processing on plant-based foods and phytochemicals present in them. Harsher processing techniques, such as protein extraction, resulted in the loss of phytochemicals from the raw material, while fermentation increased their abundances. Additionally, fermentation increased the abundances of phytochemicals with possibly higher bioavailability, as was demonstrated with isoflavonoids. By selecting proper fermentation raw materials, cultures, and conditions, it could be used to produce fermented plant-based foods and beverages from locally grown protein-rich sources, such as lupins.

ACKNOWLEDGEMENTS

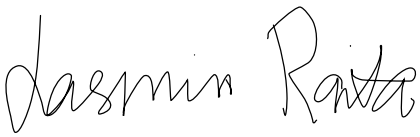
This thesis and the work involved in it was conducted at the Food Sciences unit at the University of Turku, Faculty of Technology. This research was carried out as part of the Newplant project funded by the Jane and Aatos Erkko foundation. I am deeply grateful for University of Turku Graduate School, Turku University Foundation, and HealthFerm project for financial support.

I would like to express my gratitude to my supervisors, Prof. Kati Hanhineva, Doc. Ville Koistinen, and Prof. Baoru Yang. Thank you Kati for introducing me to the world of metabolomics and for this whole opportunity to conduct this doctoral thesis. Thank you Ville for helping me with the metabolomics analysis and statistics, and for the inspiration to put effort into visualizations. Thank you Baoru for guidance throughout this journey. I am deeply grateful for my co-writers Hany Ahmed, Kang Chen, Veera Houttu, Retu Haikonen, Anna Kårlund, Maaria Kortesniemi, Malviina Nikola, Niina Kelanne, and Oskar Laaksonen. Especially the first article which was a tough one, so special thank you to everyone who provided their help and scientific knowledge to improve it. I want to thank my pre-examiners Assistant Professor Stephan Van Vliet and Associate Professor Ndegwa H. Maina for using their time and effort to comment this thesis for me to improve it.

Thank you to all the colleagues at the Food Sciences Unit and the technical staff for all of their help and support during this PhD work. Special thanks go to the members of the Food, gut, and nutritional metabolomics group.

Thanks to my family, äiti, isi, Annina, Oliver & Lenni for the support and for trying to understand what I am doing for my job. Kiitos mammalle yrityksestä ymmärtää mitä olen tehnyt työkseni.

My deepest gratitude goes to Pekka, the one who had to go through all of this with me (involuntarily :D). Lastly, the other loves of my life Björn Igor and Mörri Mau, for making my life meaningful.



Turku, April 2026

DISCLOSURE OF THE USE OF AI TOOLS

Language polishing of this doctoral thesis was supported by the AI language model ChatGPT (OpenAI). As the author, I am solely responsible for all scientific content, including the reasearch findings.

REFERENCES

1. Rockström J, Thilsted SH, Willett WC, et al. The EAT–Lancet Commission on healthy, sustainable, and just food systems. *The Lancet*. 2025;406(10512):1625-1700. doi:10.1016/S0140-6736(25)01201-2

2. Springmann M, Wiebe K, Mason-D'Croz D, Sulser TB, Rayner M, Scarborough P. Health and nutritional aspects of sustainable diet strategies and their association with environmental impacts: a global modelling analysis with country-level detail. *Lancet Planet Health*. 2018;2(10):e451-e461. doi:10.1016/S2542-5196(18)30206-7

3. Willett W, Rockström J, Loken B, et al. Food in the Anthropocene: the EAT-Lancet Commission on healthy diets from sustainable food systems. *Lancet Lond Engl*. 2019;393(10170):447-492. doi:10.1016/S0140-6736(18)31788-4

4. Touvier M, Louzada ML da C, Mozaffarian D, Baker P, Juul F, Srour B. Ultra-processed foods and cardiometabolic health: public health policies to reduce consumption cannot wait. *BMJ*. 2023;383:e075294. doi:10.1136/bmj-2023-075294

5. Qu Y, Hu W, Huang J, et al. Ultra-processed food consumption and risk of cardiovascular events: a systematic review and dose-response meta-analysis. *eClinicalMedicine*. 2024;69:102484. doi:10.1016/j.eclinm.2024.102484

6. Beal T, Manohar S, Miachon L, Fanzo J. Nutrient-dense foods and diverse diets are important for ensuring adequate nutrition across the life course. *Proc Natl Acad Sci*. 2024;121(50):e2319007121. doi:10.1073/pnas.2319007121

7. Springmann M, Clark M, Mason-D'Croz D, et al. Options for keeping the food system within environmental limits. *Nature*. 2018;562(7728):7728. doi:10.1038/s41586-018-0594-0

8. Kim J, Wilkens LR, Haiman CA, Le Marchand L, Park SY. Plant-based dietary patterns and mortality from all causes, cardiovascular disease, and cancer: The Multiethnic Cohort Study. *Clin Nutr*. 2024;43(6):1447-1453. doi:10.1016/j.clnu.2024.04.035

9. Prioux C, Wagner S, Fézeu LK, et al. Cross-sectional associations between healthy and unhealthy plant-based diets and metabolic syndrome in three distinct French populations: a meta-analysis. *Br J Nutr*. 2025;133(7):949-965. doi:10.1017/S0007114525000376

10. VanCleef TR, Hutchens A. Plant-Based Diets and Cardiovascular Disease in Older Adults: An Integrative Literature Review. *Biol Res Nurs*. 2025;27(1):109-126. doi:10.1177/10998004241268287

11. Leziak A, Lipina J, Reclik M, et al. Dietary Modulation of Metabolic Health: From Bioactive Compounds to Personalized Nutrition. *Metabolites*. 2025;15(9). doi:10.3390/metabo15090624

12. Barabasi AL, Menichetti G, Loscalzo J. The unmapped chemical complexity of our diet. *Nat Food*. 2019;1. doi:10.1038/s43016-019-0005-1

13. Kumar N, Goel N. Phenolic acids: Natural versatile molecules with promising therapeutic applications. *Biotechnol Rep*. 2019;24:e00370. doi:10.1016/j.btre.2019.e00370

14. Shen N, Wang T, Gan Q, Liu S, Wang L, Jin B. Plant flavonoids: Classification, distribution, biosynthesis, and antioxidant activity. *Food Chem*. 2022;383:132531. doi:10.1016/j.foodchem.2022.132531

15. Curtain F, Grafenauer S. Plant-Based Meat Substitutes in the Flexitarian Age: An Audit of Products on Supermarket Shelves. *Nutrients*. 2019;11(11):2603. doi:10.3390/nu11112603

16. Şanlier N, Gökcen BB, Sezgin AC. Health benefits of fermented foods. *Crit Rev Food Sci Nutr*. 2019;59(3):506-527. doi:10.1080/10408398.2017.1383355

17. Muszalska A, Wiecanowska J, Michałowska J, et al. The Role of the Planetary Diet in Managing Metabolic Syndrome and Cardiovascular Disease: A Narrative Review. *Nutrients*. 2025;17(5):862. doi:10.3390/nu17050862

18. Päivärinta E, Itkonen ST, Pellinen T, et al. Replacing Animal-Based Proteins with Plant-Based Proteins Changes the Composition of a Whole Nordic Diet—A Randomised Clinical Trial in Healthy Finnish Adults. *Nutrients*. 2020;12(4). doi:10.3390/nu12040943

19. Najjar RS, Moore CE, Montgomery BD. A defined, plant-based diet utilized in an outpatient cardiovascular clinic effectively treats hypercholesterolemia and hypertension and reduces medications. *Clin Cardiol*. 2018;41(3):307-313. doi:10.1002/clc.22863

20. Thaiudom S, Posridee K, Liangchawengwong S, et al. Plant-Based Diet for Glycemic Control, Insulin Sensitivity, and Lipid Profile in Type 2 Diabetes: A Systematic Review. *Foods*. 2025;14(11):1919. doi:10.3390/foods14111919

21. Hanick CJ, Peterson CM, Davis BC, Sabaté J, Kelly JH. A whole-food, plant-based intensive lifestyle intervention improves glycaemic control and reduces medications in individuals with type 2 diabetes: a randomised controlled trial. *Diabetologia*. 2025;68(2):308-319. doi:10.1007/s00125-024-06272-8

22. Lee H, Lee JE, Kang M. Association of plant-based diet indices with all-cause and cause-specific mortality among Korean adults in the KNHANES. *Sci Rep*. 2025;15(1):41007. doi:10.1038/s41598-025-24698-x

23. Damodaran S, Parkin KL. *Fennema's Food Chemistry*. Vol 2017. 5th edition. CRC Press; 2017.

24. Ettinger L, Falkeisen A, Knowles S, et al. Consumer Perception and Acceptability of Plant-Based Alternatives to Chicken. *Foods*. 2022;11(15):15. doi:10.3390/foods11152271

25. Sha L, Xiong YL. Plant protein-based alternatives of reconstructed meat: Science, technology, and challenges. *Trends Food Sci Technol*. 2020;102:51-61. doi:10.1016/j.tifs.2020.05.022

26. Melville H, Shahid M, Gaines A, et al. The nutritional profile of plant-based meat analogues available for sale in Australia. *Nutr Diet*. 2023;80(2):211-222. doi:10.1111/1747-0080.12793

27. van Vliet S, Bain JR, Muehlbauer MJ, et al. A metabolomics comparison of plant-based meat and grass-fed meat indicates large nutritional differences despite comparable Nutrition Facts panels. *Sci Rep*. 2021;11(1):13828. doi:10.1038/s41598-021-93100-3

28. Harnack L, Mork S, Valluri S, et al. Nutrient Composition of a Selection of Plant-Based Ground Beef Alternative Products Available in the United States. *J Acad Nutr Diet*. Published online June 2, 2021:S2212-2672(21)00303-8. doi:10.1016/j.jand.2021.05.002

29. Mayer Labba IC, Steinhausen H, Almius L, Bach Knudsen KE, Sandberg AS. Nutritional Composition and Estimated Iron and Zinc Bioavailability of Meat Substitutes Available on the Swedish Market. *Nutrients*. 2022;14(19):19. doi:10.3390/nu14193903

30. Yang Y, Zheng Y, Ma W, Zhang Y, Sun C, Fang Y. Meat and plant-based meat analogs: Nutritional profile and *in vitro* digestion comparison. *Food Hydrocoll*. 2023;143:108886. doi:10.1016/j.foodhyd.2023.108886

31. Abdullah FAA, Dordevic D, Kabourkova E, Zemancová J, Dordevic S. Antioxidant and Sensorial Properties: Meat Analogues versus Conventional Meat Products. *Processes*. 2022;10(9):9. doi:10.3390/pr10091864

32. Alessandrini R, Brown MK, Pombo-Rodrigues S, Bhageerutty S, He FJ, MacGregor GA. Nutritional Quality of Plant-Based Meat Products Available in the UK: A Cross-Sectional Survey. *Nutrients*. 2021;13(12):12. doi:10.3390/nu13124225

33. Sharma N, Yeasmen N, Dube L, Orsat V. Rise of Plant-Based Beverages: A Consumer-Driven Perspective. *Food Rev Int*. 2024;0(0):1-27. doi:10.1080/87559129.2024.2351920

34. Flom JD, Sicherer SH. Epidemiology of Cow's Milk Allergy. *Nutrients*. 2019;11(5):1051. doi:10.3390/nu11051051

35. Meoni G, Sousa I, Tenori L, et al. A metabolic profiling approach to characterize and discriminate plant-based beverages and milk. *J Dairy Sci*. Published online April 17, 2025. doi:10.3168/jds.2025-26332

36. Bieck K, Ebert F, Grune T, Raupbach J. Maillard reaction products in plant-based dairy alternatives and their release during simulated gastrointestinal digestion. *Curr Res Food Sci*. 2025;10:100994. doi:10.1016/j.crfs.2025.100994

37. Pucci M, Akıllıoğlu HG, Bevilacqua M, Abate G, Lund MN. Investigation of Maillard reaction products in plant-based milk alternatives. *Food Res Int*. 2024;198:115418. doi:10.1016/j.foodres.2024.115418

38. Kersting M, Kalhoff H, Zahn K, Belgardt AJ, Sinningen K, Lücke T. Replacing cow's milk with plant-based drinks: consequences for nutrient intake of young children on

a balanced diet in Germany. *J Health Popul Nutr*. 2025;44(1):93. doi:10.1186/s41043-025-00836-z

39. Ge J, Sun CX, Corke H, Gul K, Gan RY, Fang Y. The health benefits, functional properties, modifications, and applications of pea (Pisum sativum L.) protein: Current status, challenges, and perspectives. *Compr Rev Food Sci Food Saf*. 2020;19(4):1835-1876. doi:10.1111/1541-4337.12573

40. Sá AGA, Wan Z, Jha AB, Gali KK, Warkentin TD, House JD. Influence of different amino acid scoring patterns on the protein quality of field peas. *J Food Compos Anal*. 2024;127:105938. doi:10.1016/j.jfca.2023.105938

41. Sim SYJ, Karwe MV, Moraru CI. High pressure structuring of pea protein concentrates. *J Food Process Eng*. 2019;42(7):e13261. doi:10.1111/jfpe.13261

42. Anwar D, El-Chaghaby G. NUTRITIONAL QUALITY, AMINO ACID PROFILES, PROTEIN DIGESTIBILITY CORRECTED AMINO ACID SCORES AND ANTIOXIDANT PROPERTIES OF FRIED TOFU AND SEITAN. *Food Environ Saf J*. 2019;18(3):3. Accessed March 20, 2025. https://fens.usv.ro/index.php/FENS/article/view/657

43. Kumar P, Chatli MK, Mehta N, Singh P, Malav OP, Verma AK. Meat analogues: Health promising sustainable meat substitutes. *Crit Rev Food Sci Nutr*. 2017;57(5):923-932. doi:10.1080/10408398.2014.939739

44. Banach JL, van der Berg JP, Kleter G, et al. Alternative proteins for meat and dairy replacers: Food safety and future trends. *Crit Rev Food Sci Nutr*. 2022;0(0):1-18. doi:10.1080/10408398.2022.2089625

45. Zhao D, Huang L, Li H, et al. Ingredients and Process Affect the Structural Quality of Recombinant Plant-Based Meat Alternatives and Their Components. *Foods*. 2022;11(15):2202. doi:10.3390/foods11152202

46. Liu K. *Soybeans: Chemistry, Technology, and Utilization*. Springer US; 1997. doi:10.1007/978-1-4615-1763-4

47. Wang T, Qin GX, Sun ZW, Zhao Y. Advances of Research on Glycinin and β-Conglycinin: A Review of Two Major Soybean Allergenic Proteins. *Crit Rev Food Sci Nutr*. 2014;54(7):850-862. doi:10.1080/10408398.2011.613534

48. Developing soy production in central and northern Europe. Accessed July 2, 2024. https://www.cabidigitallibrary.org/doi/epdf/10.1079/9781780644981.0109

49. Jenkins DJA, Mirrahimi A, Srichaikul K, et al. Soy Protein Reduces Serum Cholesterol by Both Intrinsic and Food Displacement Mechanisms, ,. *J Nutr*. 2010;140(12):2302S-2311S. doi:10.3945/jn.110.124958

50. Balentine DA. Qualified Health Claim Petition – Soybean Oil and Reduced Risk of Coronary Heart Disease (Docket No. FDA-2016-Q-0995). Published online 2016.

51. West LM, Sabaté J, Nwachukwu ID, et al. Effects of Fermented Soy on Cognition in Older Adults:

Outcomes of a Randomized, Controlled Trial. *Nutrients*. 2025;17(18):2936. doi:10.3390/nu17182936

52. Kojima A, Ikehara S, Kamiya K, et al. Natto Intake is Inversely Associated with Osteoporotic Fracture Risk in Postmenopausal Japanese Women. *J Nutr*. 2020;150(3):599-605. doi:10.1093/jn/nxz292

53. Raita J, Ahmed H, Chen K, et al. Existing food processing classifications overlook the phytochemical composition of processed plant-based protein-rich foods. *Nat Food*. Published online March 24, 2025:1-10. doi:10.1038/s43016-025-01148-5

54. Lucas MM, Stoddard FL, Annicchiarico P, et al. The future of lupin as a protein crop in Europe. *Front Plant Sci*. 2015;6. doi:10.3389/fpls.2015.00705

55. Taberner-Pibernat P, Ribas-Agustí A, Quinteros G, et al. Compositional and nutritional value of lupin cultivars: Identifying high-protein seeds for enhanced protein isolate production and phytochemical valorisation. *J Agric Food Res*. 2025;22:102010. doi:10.1016/j.jafr.2025.102010

56. Cortés-Avendaño P, Tarvainen M, Suomela JP, Glorio-Paulet P, Yang B, Repo-Carrasco-Valencia R. Profile and Content of Residual Alkaloids in Ten Ecotypes of Lupinus mutabilis Sweet after Aqueous Debittering Process. *Plant Foods Hum Nutr*. 2020;75(2):184-191. doi:10.1007/s11130-020-00799-y

57. Erbaş M, Certel M, Uslu MK. Some chemical properties of white lupin seeds (*Lupinus albus* L.). *Food Chem*. 2005;89(3):341-345. doi:10.1016/j.foodchem.2004.02.040

58. Gdala J, Buraczewska L. Chemical composition and carbohydrate content of seeds from several lupin species. *J Anim Feed Sci*. 1996;05(4):403-416.

59. Olukomaiya OO, Adiamo OQ, Fernando WC, Mereddy R, Li X, Sultanbawa Y. Effect of solid-state fermentation on proximate composition, anti-nutritional factor, microbiological and functional properties of lupin flour. *Food Chem*. 2020;315:126238. doi:10.1016/j.foodchem.2020.126238

60. Fang L, Wang W, Dou Z, et al. Effects of mixed fermentation of different lactic acid bacteria and yeast on phytic acid degradation and flavor compounds in sourdough. *LWT*. 2023;174:114438. doi:10.1016/j.lwt.2023.114438

61. Elhordoy MR, Kadam A, Vazquez D, Medrano A, Koksel F. Exploring the Potential of Lupin (Lupinus angustifolius) Flour-Based Ingredients in Developing High Moisture Meat Analogues. *J Food Sci*. 2025;90(7):e70407. doi:10.1111/1750-3841.70407

62. Arnoldi A, Boschin G, Zanoni C, Lammi C. The health benefits of sweet lupin seed flours and isolated proteins. *J Funct Foods*. 2015;18:550-563. doi:10.1016/j.jff.2015.08.012

63. Bähr M, Fechner A, Krämer J, Kiehntopf M, Jahreis G. Lupin protein positively affects plasma LDL cholesterol and LDL:HDL cholesterol ratio in hypercholesterolemic adults after four weeks of supplementation: a

randomized, controlled crossover study. *Nutr J*. 2013;12(1):107. doi:10.1186/1475-2891-12-107

64. Pavanello C, Lammi C, Ruscica M, et al. Effects of a lupin protein concentrate on lipids, blood pressure and insulin resistance in moderately dyslipidaemic patients: A randomised controlled trial. *J Funct Foods*. 2017;37:8-15. doi:10.1016/j.jff.2017.07.039

65. Bryant L, Rangan A, Grafenauer S. Lupins and Health Outcomes: A Systematic Literature Review. *Nutrients*. 2022;14(2):327. doi:10.3390/nu14020327

66. Vaughan MM, Block A, Christensen SA, Allen LH, Schmelz EA. The effects of climate change associated abiotic stresses on maize phytochemical defenses. *Phytochem Rev*. 2018;17(1):37-49. doi:10.1007/s11101-017-9508-2

67. Karaś M, Jakubczyk A, Szymanowska U, Złotek U, Zielińska E. Digestion and bioavailability of bioactive phytochemicals. *Int J Food Sci Technol*. 2017;52(2):291-305. doi:10.1111/ijfs.13323

68. Lingua MS, Wunderlin DA, Baroni MV. Effect of simulated digestion on the phenolic components of red grapes and their corresponding wines. *J Funct Foods*. 2018;44:86-94. doi:10.1016/j.jff.2018.02.034

69. Lippolis T, Cofano M, Caponio GR, et al. Bioaccessibility and Bioavailability of Diet Polyphenols and Their Modulation of Gut Microbiota. *Int J Mol Sci*. 2023;24(4). doi:10.3390/ijms24043813

70. Hu Y, Lin Q, Zhao H, et al. Bioaccessibility and bioavailability of phytochemicals: Influencing factors, improvements, and evaluations. *Food Hydrocoll*. 2023;135:108165. doi:10.1016/j.foodhyd.2022.108165

71. Koistinen VM, Tuomainen M, Lehtinen P, et al. Side-stream products of malting: a neglected source of phytochemicals. *Npj Sci Food*. 2020;4(1):21. doi:10.1038/s41538-020-00081-0

72. Hsiao YH, Ho CT, Pan MH. Bioavailability and health benefits of major isoflavone aglycones and their metabolites. *J Funct Foods*. 2020;74:104164. doi:10.1016/j.jff.2020.104164

73. Abd Allah MA, Khiralla G, Elhariry H. Evaluation of bioaccessibility of bioactive compounds in ready-to-eat refrigerated and frozen broccoli using in vitro digestion models. *Sci Rep*. 2025;15(1):41457. doi:10.1038/s41598-025-26034-9

74. Multari S, Neacsu M, Scobbie L, et al. Nutritional and Phytochemical Content of High-Protein Crops. *J Agric Food Chem*. 2016;64(41):7800-7811. doi:10.1021/acs.jafc.6b00926

75. Fernandez-Pan I, Horvitz S, Ibañez FC, Arroqui C, Beriain MJ, Virseda P. Extra-virgin olive oil enriched with lycopene: From industrial tomato by-products to consumer. *Food Sci Nutr*. 2024;12(8):5815-5823. doi:10.1002/fsn3.4224

76. Zavala-López M, Flint-García S, García-Lara S. Compositional Variation in Trans-Ferulic, p-coumaric, and Diferulic Acids Levels Among Kernels of Modern and Traditional Maize (Zea mays L.) Hybrids. *Front Nutr*. 2020;7. doi:10.3389/fnut.2020.600747

77. Maity S, Kinra M, Nampoothiri M, Arora D, Pai KSR, Mudgal J. Caffeic acid, a dietary polyphenol, as a promising candidate for combination therapy. *Chem Pap*. 2022;76(3):1271-1283. doi:10.1007/s11696-021-01947-7

78. Amiot MJ, Latgé C, Plumey L, Raynal S. Intake Estimation of Phytochemicals in a French Well-Balanced Diet. *Nutrients*. 2021;13(10):3628. doi:10.3390/nu13103628

79. Crowe-White KM, Evans LW, Kuhnle GGC, et al. Flavan-3-ols and Cardiometabolic Health: First Ever Dietary Bioactive Guideline. *Adv Nutr*. 2022;13(6):2070-2083. doi:10.1093/advances/nmac105

80. Zamora-Ros R, Biessy C, Rothwell JA, et al. Dietary polyphenol intake and their major food sources in the Mexican Teachers' Cohort. *Br J Nutr*. 2018;120(3):353-360. doi:10.1017/S0007114518001381

81. Zamora-Ros R, Knaze V, Rothwell JA, et al. Dietary polyphenol intake in Europe: the European Prospective Investigation into Cancer and Nutrition (EPIC) study. *Eur J Nutr*. 2016;55(4):1359-1375. doi:10.1007/s00394-015-0950-x

82. Clarke ED, Rollo ME, Collins CE, et al. The Relationship between Dietary Polyphenol Intakes and Urinary Polyphenol Concentrations in Adults Prescribed a High Vegetable and Fruit Diet. *Nutrients*. 2020;12(11). doi:10.3390/nu12113431

83. Burkholder-Cooley N, Rajaram S, Haddad E, Fraser GE, Jaceldo-Siegl K. Comparison of polyphenol intakes according to distinct dietary patterns and food sources in the Adventist Health Study-2 cohort. *Br J Nutr*. 2016;115(12):2162-2169. doi:10.1017/S0007114516001331

84. Roberts K. *Handbook of Plant Science*. Vol 2017. John Wiley & Sons; 2007.

85. Parmenter BH, Thompson AS, Bondonno NP, et al. High diversity of dietary flavonoid intake is associated with a lower risk of all-cause mortality and major chronic diseases. *Nat Food*. 2025;6(7):668-680. doi:10.1038/s43016-025-01176-1

86. Yang Q, Wang G. Isoflavonoid metabolism in leguminous plants: an update and perspectives. *Front Plant Sci*. 2024;15. doi:10.3389/fpls.2024.1368870

87. Dastmalchi M, Dhaubhadel S. Soybean Seed Isoflavonoids: Biosynthesis and Regulation. In: Jetter R, ed. *Phytochemicals – Biosynthesis, Function and Application: Volume 44*. Springer International Publishing; 2014:1-21. doi:10.1007/978-3-319-04045-5_1

88. Xu Q, Hu X, Cui S, et al. Genome-Wide Identification and Characterization of Isoflavone Synthase (IFS) Gene Family, and Analysis of GgARF4-GgIFS9 Regulatory Module in Glycyrrhiza glabra. *Int J Mol Sci*. 2025;26(21). doi:10.3390/ijms262110435

89. Baky MH, Elshahed M, Wessjohann L, Farag MA. Interactions between dietary flavonoids and the gut microbiome: a comprehensive review. *Br J Nutr*. 2022;128(4):577-591. doi:10.1017/S0007114521003627

90. Yuan JP, Wang JH, Liu X. Metabolism of dietary soy isoflavones to equol by human intestinal microflora – implications

for health. *Mol Nutr Food Res*. 2007;51(7):765-781. doi:10.1002/mnfr.200600262

91. Jackson RL, Greiwe JS, Schwen RJ. Emerging evidence of the health benefits of S-equol, an estrogen receptor β agonist. *Nutr Rev*. 2011;69(8):432-448. doi:10.1111/j.1753-4887.2011.00400.x

92. Miklos A, Tero-Vescan A, Muntean D. Isoflavonoids − Dual Action on Estrogen Receptors. *Acta Biol Marisiensis*. 2018;1:61-67. doi:10.2478/abmj-2018-0014

93. Han RM, Tian YX, Liu Y, et al. Comparison of Flavonoids and Isoflavonoids as Antioxidants. *J Agric Food Chem*. 2009;57(9):3780-3785. doi:10.1021/jf803850p

94. Hämäläinen M, Nieminen R, Vuorela P, Heinonen M, Moilanen E. Anti-Inflammatory Effects of Flavonoids: Genistein, Kaempferol, Quercetin, and Daidzein Inhibit STAT-1 and NF-κB Activations, Whereas Flavone, Isorhamnetin, Naringenin, and Pelargonidin Inhibit only NF-κB Activation along with Their Inhibitory Effect on iNOS Expression and NO Production in Activated Macrophages. *Mediators Inflamm*. 2007;2007(1):045673. doi:10.1155/2007/45673

95. Pawlowski JW, Martin BR, McCabe GP, et al. Impact of equol-producing capacity and soy-isoflavone profiles of supplements on bone calcium retention in postmenopausal women: a randomized crossover trial12. *Am J Clin Nutr*. 2015;102(3):695-703. doi:10.3945/ajcn.114.093906

96. Yamamoto S, Sobue T, Kobayashi M, Sasaki S, Tsugane S, For the Japan Public Health Center-Based Prospective Study on Cancer Cardiovascular Diseases (JPHC Study) Group. Soy, Isoflavones, and Breast Cancer Risk in Japan. *JNCI J Natl Cancer Inst*. 2003;95(12):906-913. doi:10.1093/jnci/95.12.906

97. Intharuksa A, Arunotayanun W, Takuathung MN, et al. Daidzein and Genistein: Natural Phytoestrogens with Potential Applications in Hormone Replacement Therapy. *Int J Mol Sci*. 2025;26(14). doi:10.3390/ijms26146973

98. Cushnie TPT, Cushnie B, Lamb AJ. Alkaloids: An overview of their antibacterial, antibiotic-enhancing and antivirulence activities. *Int J Antimicrob Agents*. 2014;44(5):377-386. doi:10.1016/j.ijantimicag.2014.06.001

99. Schläger S, Dräger B. Exploiting plant alkaloids. *Curr Opin Biotechnol*. 2016;37:155-164. doi:10.1016/j.copbio.2015.12.003

100. Prakash AS, Pereira TN, Reilly PEB, Seawright AA. Pyrrolizidine alkaloids in human diet. *Mutat Res Toxicol Environ Mutagen*. 1999;443(1):53-67. doi:10.1016/S1383-5742(99)00010-1

101. Wiedenfeld H, Edgar J. Toxicity of pyrrolizidine alkaloids to humans and ruminants. *Phytochem Rev*. 2011;10(1):137-151. doi:10.1007/s11101-010-9174-0

102. de Cortes Sánchez M, Altares P, Pedrosa MM, et al. Alkaloid variation during germination in different lupin species. *Food Chem*.

2005;90(3):347-355. doi:10.1016/j.foodchem.2004.04.008

103. Chain (CONTAM) EP on C in the F, Schrenk D, Bodin L, et al. Scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids in feed and food, in particular in lupins and lupin-derived products. *EFSA J*. 2019;17(11):e05860. doi:10.2903/j.efsa.2019.5860

104. Bhambhani S, Kondhare KR, Giri AP. Diversity in Chemical Structures and Biological Properties of Plant Alkaloids. *Molecules*. 2021;26(11):3374. doi:10.3390/molecules26113374

105. Debnath B, Singh WS, Das M, et al. Role of plant alkaloids on human health: A review of biological activities. *Mater Today Chem*. 2018;9:56-72. doi:10.1016/j.mtchem.2018.05.001

106. Schrenk D, Bodin L, Chipman JK, et al. Scientific opinion on the risks for animal and human health related to the presence of quinolizidine alkaloids in feed and food, in particular in lupins and lupin-derived products. *EFSA J*. 2019;17(11):e05860. doi:10.2903/j.efsa.2019.5860

107. Robbins RJ. Phenolic Acids in Foods: An Overview of Analytical Methodology. *J Agric Food Chem*. 2003;51(10):2866-2887. doi:10.1021/jf026182t

108. Khalil M i., Alam N, Moniruzzaman M, Sulaiman S a., Gan S h. Phenolic Acid Composition and Antioxidant Properties of Malaysian Honeys. *J Food Sci*. 2011;76(6):C921-C928. doi:10.1111/j.1750-3841.2011.02282.x

109. Mattila P, Kumpulainen J. Determination of Free and Total Phenolic Acids in Plant-Derived Foods by HPLC with Diode-Array Detection. *J Agric Food Chem*. 2002;50(13):3660-3667. doi:10.1021/jf020028p

110. Liu J, Du C, Beaman HT, et al. Characterization of Phenolic Acid Antimicrobial and Antioxidant Structure–Property Relationships. *Pharmaceutics*. 2020;12(5). doi:10.3390/pharmaceutics12050419

111. Hole AS, Rud I, Grimmer S, Sigl S, Narvhus J, Sahlstrøm S. Improved Bioavailability of Dietary Phenolic Acids in Whole Grain Barley and Oat Groat following Fermentation with Probiotic Lactobacillus acidophilus, Lactobacillus johnsonii, and Lactobacillus reuteri. *J Agric Food Chem*. 2012;60(25):6369-6375. doi:10.1021/jf300410h

112. Day L, Cakebread JA, Loveday SM. Food proteins from animals and plants: Differences in the nutritional and functional properties. *Trends Food Sci Technol*. 2022;119:428-442. doi:10.1016/j.tifs.2021.12.020

113. Coultate T. *Food The Chemistry of Its Components*. Vol 2016. The Royal Society of Chemistry; 2016.

114. Wanders AJ, Heerschop SN, Biesbroek S, Dötsch-Klerk M. Replacing Animal Meat with Plant-Based Meat Alternatives: The Impact of Protein Quality on Protein Adequacy in the Dutch Diet. *Curr Dev Nutr*. 2025;9(3):104562. doi:10.1016/j.cdnut.2025.104562

115. De Marchi M, Costa A, Pozza M, Goi A, Manuelian CL. Detailed characterization of plant-based

burgers. *Sci Rep*. 2021;11(1):1. doi:10.1038/s41598-021-81684-9

116. Cao H, Wang X, Wu W, et al. Exploring the influence of lysine incorporation on the physicochemical properties of quinoa protein gels formed under microwave versus conventional heating conditions. *Food Res Int*. 2025;202:115678. doi:10.1016/j.foodres.2025.115678

117. Xie Y, Cai L, Zhao D, et al. Real meat and plant-based meat analogues have different in vitro protein digestibility properties. *Food Chem*. 2022;387:132917. doi:10.1016/j.foodchem.2022.132917

118. Cutroneo S, Prandi B, Faccini A, Pellegrini N, Sforza S, Tedeschi T. Comparison of protein quality and digestibility between plant-based and meat-based burgers. *Food Res Int*. 2023;172:113183. doi:10.1016/j.foodres.2023.113183

119. Zeinatulina A, Tanilas K, Ehala-Aleksejev K, Viiard E, Kriščiunaite T. Digestibility of protein and estimated bioavailability of mineral compounds in plant-based yoghurt alternatives. *Future Foods*. 2025;11:100545. doi:10.1016/j.fufo.2025.100545

120. Popova A, Mihaylova D. Antinutrients in Plant-based Foods: A Review. *Open Biotechnol J*. 2019;13(1). doi:10.2174/1874070701913010068

121. Adamcová A, Laursen KH, Ballin NZ. Lectin Activity in Commonly Consumed Plant-Based Foods: Calling for Method Harmonization and Risk Assessment. *Foods*. 2021;10(11):11. doi:10.3390/foods10112796

122. Vidal-Valverde C, Frias J, Diaz-Pollan C, Fernandez M, Lopez-Jurado M, Urbano G. Influence of Processing on Trypsin Inhibitor Activity of Faba Beans and Its Physiological Effect. *J Agric Food Chem*. 1997;45(9):3559-3564. doi:10.1021/jf9700236

123. Adamidou S, Nengas I, Grigorakis K, Nikolopoulou D, Jauncey K. Chemical Composition and Antinutritional Factors of Field Peas (Pisum sativum), Chickpeas (Cicer arietinum), and Faba Beans (Vicia faba) as Affected by Extrusion Preconditioning and Drying Temperatures. *Cereal Chem*. 2011;88(1):80-86. doi:10.1094/CCHEM-05-10-0077

124. Kumar AYN, Chowdhury A, Kumar R, Maurya VK, Batabyal S, Ghosh M. Impact of microwave processing on phytochemicals, antioxidant status, anti-nutritional factors and metabolite profile of maize flour. *Appl Food Res*. 2025;5(1):100712. doi:10.1016/j.afres.2025.100712

125. Eskin M, Shahidi F. *Biochemistry of Foods*. Vol 2013. 3rd edition. Academic Press; 2013.

126. Halász A, Baráth Á, Simon-Sarkadi L, Holzapfel W. Biogenic amines and their production by microorganisms in food. *Trends Food Sci Technol*. 1994;5(2):42-49. doi:10.1016/0924-2244(94)90070-1

127. Liu Y, Luo L, Huang G, Zhou K, Xu ZL. Visual detection of biogenic amines in foods - Current technologies, challenges, and future prospects. *Trends Food Sci Technol*. 2025;161:105049. doi:10.1016/j.tifs.2025.105049

128. Shalaby AR. Significance of biogenic amines to food safety and human health. *Food Res Int*. 1996;29(7):675-690. doi:10.1016/S0963-9969(96)00066-X

129. Elhalis H, See XY, Osen R, Chin XH, Chow Y. Significance of Fermentation in Plant-Based Meat Analogs: A Critical Review of Nutrition, and Safety-Related Aspects. *Foods*. 2023;12(17):3222. doi:10.3390/foods12173222

130. Wang Y, Liu Y, Huang X, et al. A Review on Mechanistic Overview on the Formation of Toxic Substances during the Traditional Fermented Food Processing. *Food Rev Int*. 2023;39(3):1275-1292. doi:10.1080/87559129.2021.1933021

131. Gillman PK. Monoamine oxidase inhibitors: A review concerning dietary tyramine and drug interactions. Published online 2019.

132. Madeo F, Carmona-Gutierrez D, Kepp O, Kroemer G. Spermidine delays aging in humans. *Aging*. 2018;10(8):2209-2211. doi:10.18632/aging.101517

133. Spano G, Russo P, Lonvaud-Funel A, et al. Biogenic amines in fermented foods. *Eur J Clin Nutr*. 2010;64(3):S95-S100. doi:10.1038/ejcn.2010.218

134. Naila A, Flint S, Fletcher G, Bremer P, Meerdink G. Control of Biogenic Amines in Food—Existing and Emerging Approaches. *J Food Sci*. 2010;75(7):R139-R150. doi:10.1111/j.1750-3841.2010.01774.x

135. Augustin MA, Riley M, Stockmann R, et al. Role of food processing in food and nutrition security. *Trends Food Sci Technol*. 2016;56:115-125. doi:10.1016/j.tifs.2016.08.005

136. Chung IM, Seo SH, Ahn JK, Kim SH. Effect of processing, fermentation, and aging treatment to content and profile of phenolic compounds in soybean seed, soy curd and soy paste. *Food Chem*. 2011;127(3):960-967. doi:10.1016/j.foodchem.2011.01.065

137. Lee S, Lee J. Effects of oven-drying, roasting, and explosive puffing process on isoflavone distributions in soybeans. *Food Chem*. 2009;112(2):316-320. doi:10.1016/j.foodchem.2008.05.065

138. Nolden AA, Forde CG. The Nutritional Quality of Plant-Based Foods. *Sustainability*. 2023;15(4):4. doi:10.3390/su15043324

139. Lin S, Huff H e., Hsieh F. Extrusion Process Parameters, Sensory Characteristics, and Structural Properties of a High Moisture Soy Protein Meat Analog. *J Food Sci*. 2002;67(3):1066-1072. doi:10.1111/j.1365-2621.2002.tb09454.x

140. Lazou AE. Food extrusion: An advanced process for innovation and novel product development. *Crit Rev Food Sci Nutr*. 2024;64(14):4532-4560. doi:10.1080/10408398.2022.2143474

141. Pietsch VL, Werner R, Karbstein HP, Emin MA. High moisture extrusion of wheat gluten: Relationship between process parameters, protein polymerization, and final product characteristics. *J Food Eng*. 2019;259:3-11. doi:10.1016/j.jfoodeng.2019.04.006

142. Chen FL, Wei YM, Zhang B, Ojokoh AO. System parameters and product properties response of soybean protein extruded at wide moisture range. *J Food Eng*. 2010;96(2):208-213. doi:10.1016/j.jfoodeng.2009.07.014

143. Kristiawan M, Micard V, Maladira P, et al. Multi-scale structural changes of starch and proteins during pea flour extrusion. *Food Res Int*. 2018;108:203-215. doi:10.1016/j.foodres.2018.03.027

144. Ai Y, Cichy KA, Harte JB, Kelly JD, Ng PKW. Effects of extrusion cooking on the chemical composition and functional properties of dry common bean powders. *Food Chem*. 2016;211:538-545. doi:10.1016/j.foodchem.2016.05.095

145. Fellows PJ. *Food Processing Technology*. Vol 2009. 3rd edition. Woodhead Publishing Series in Food Science, Technology and Nutrition; 2009.

146. Nisov A, Nikinmaa M, Nordlund E, Sozer N. Effect of pH and temperature on fibrous structure formation of plant proteins during high-moisture extrusion processing. *Food Res Int*. 2022;156:111089. doi:10.1016/j.foodres.2022.111089

147. Lazou A, Krokida M. Structural and textural characterization of corn–lentil extruded snacks. *J Food Eng*. 2010;100(3):392-408. doi:10.1016/j.jfoodeng.2010.04.024

148. Osen R, Toelstede S, Wild F, Eisner P, Schweiggert-Weisz U. High moisture extrusion cooking of pea protein isolates: Raw material characteristics, extruder responses, and texture properties. *J Food Eng*. 2014;127:67-74. doi:10.1016/j.jfoodeng.2013.11.023

149. Orozco-Angelino X, Espinosa-Ramírez J, Serna-Saldívar SO. Extrusion as a tool to enhance the nutritional and bioactive potential of cereal and legume by-products. *Food Res Int*. 2023;169:112889. doi:10.1016/j.foodres.2023.112889

150. Bamforth CW. *Food, Fermentation and Micro-Organisms*. Vol 2005. Blackwell Publishing; 2005.

151. Romulo A, Surya R. Tempe: A traditional fermented food of Indonesia and its health benefits. *Int J Gastron Food Sci*. 2021;26:100413. doi:10.1016/j.ijgfs.2021.100413

152. Fernandez Castaneda LA, Auer J, Leong S lin L, et al. Optimizing Soaking and Boiling Time in the Development of Tempeh-like Products from Faba Bean (Vicia faba L.). *Fermentation*. 2024;10(8):407. doi:10.3390/fermentation10080407

153. Yeo SK, Ewe JA. Effect of fermentation on the phytochemical contents and antioxidant properties of plant foods. In: *Advances in Fermented Foods and Beverages*. Elsevier; 2015:107-122. doi:10.1016/B978-1-78242-015-6.00005-0

154. Kano M, Takayanagi T, Harada K, Sawada S, Ishikawa F. Bioavailability of Isoflavones after Ingestion of Soy Beverages in Healthy Adults. *J Nutr*. 2006;136(9):2291-2296. doi:10.1093/jn/136.9.2291

155. da Silva LH, Celeghini RMS, Chang YK. Effect of the

fermentation of whole soybean flour on the conversion of isoflavones from glycosides to aglycones. *Food Chem*. 2011;128(3):640-644. doi:10.1016/j.foodchem.2011.03.079

156. Lee CH. Lactic acid fermented foods and their benefits in Asia. *Food Control*. 1997;8(5):259-269. doi:10.1016/S0956-7135(97)00015-7

157. König H, Fröhlich J. Lactic Acid Bacteria. In: König H, Unden G, Fröhlich J, eds. *Biology of Microorganisms on Grapes, in Must and in Wine*. Springer International Publishing; 2017:3-41. doi:10.1007/978-3-319-60021-5_1

158. Salminen S, von Wright A, Ouwehand A. *Lactic Acid Bacteria Microbiological and Functional Aspects*. 3rd edition. Marcel Dekker, Inc; 2004.

159. Leroy F, De Vuyst L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol*. 2004;15(2):67-78. doi:10.1016/j.tifs.2003.09.004

160. Hazards (BIOHAZ) EP on B, Ricci A, Allende A, et al. Update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA 5: suitability of taxonomic units notified to EFSA until September 2016. *EFSA J*. 2017;15(3):e04663. doi:10.2903/j.efsa.2017.4663

161. Li R, Luo W, Liu Y, et al. The investigation on the characteristic metabolites of *Lactobacillus plantarum* RLL68 during fermentation of beverage from by-products of black tea manufacture. *Curr Res Food Sci*. 2022;5:1320-1329. doi:10.1016/j.crfs.2022.07.014

162. Di Cagno R, Filannino P, Vincentini O, Cantatore V, Cavoski I, Gobbetti M. Fermented Portulaca oleracea L. Juice: A Novel Functional Beverage with Potential Ameliorating Effects on the Intestinal Inflammation and Epithelial Injury. *Nutrients*. 2019;11(2):248. doi:10.3390/nu11020248

163. Laaksonen O, Kahala M, Marsol-Vall A, et al. Impact of lactic acid fermentation on sensory and chemical quality of dairy analogues prepared from lupine (Lupinus angustifolius L.) seeds. *Food Chem*. 2021;346:128852. doi:10.1016/j.foodchem.2020.128852

164. Flores M, Piornos JA. Fermented meat sausages and the challenge of their plant-based alternatives: A comparative review on aroma-related aspects. *Meat Sci*. 2021;182:108636. doi:10.1016/j.meatsci.2021.108636

165. Monica S, Bancalari E, Siroli L, et al. Lactic acid fermentation of non-conventional plant-based protein extract. *Food Res Int*. 2025;208:116174. doi:10.1016/j.foodres.2025.116174

166. Campus M, Corrias F, Angioni A, et al. Efficacy of a Native Microbial Starter in Promoting Table Olive Fermentation: An Industrial-Scale Trial at Controlled and Ambient Temperature. *Foods*. 2025;14(13). doi:10.3390/foods14132159

167. Capozzi V, Fragasso M, Romaniello R, Berbegal C, Russo P, Spano G. Spontaneous Food Fermentations and Potential Risks

for Human Health. *Fermentation*. 2017;3(4):4. doi:10.3390/fermentation3040049

168. Jasiak K, Amund D. Are spontaneously fermented plant-based foods potential sources of transferable antibiotic resistance genes? *Food Front*. 2022;3(1):46-55. doi:10.1002/fft2.123

169. Olagunju OF, Ezekiel OO, Ogunshe AO, Oyeyinka SA, Ijabadeniyi OA. Effects of fermentation on proximate composition, mineral profile and antinutrients of tamarind (*Tamarindus indica* L.) seed in the production of daddawa-type condiment. *LWT*. 2018;90:455-459. doi:10.1016/j.lwt.2017.12.064

170. Adugna AA, Tolesa GN, Abera S, Deme GD. Effect of grain fermentation and malting time on nutrient and anti nutrient composition of biscuits from Aksum finger millet (Eleusine coracana). *Cogent Food Agric*. 2024;10(1):2336690. doi:10.1080/23311932.2024.2336690

171. Endalew HW, Atlabachew M, Karavoltsos S, et al. Effect of fermentation on nutrient composition, antinutrients, and mineral bioaccessibility of finger millet based *Injera*: A traditional Ethiopian food. *Food Res Int*. 2024;190:114635. doi:10.1016/j.foodres.2024.114635

172. Luo Y, Gu Z, Han Y, Chen Z. The impact of processing on phytic acid, in vitro soluble iron and Phy/Fe molar ratio of faba bean (Vicia faba L.). *J Sci Food Agric*. 2009;89(5):861-866. doi:10.1002/jsfa.3525

173. de Oliveira SD, de Souza EL, Araújo CM, et al. Spontaneous fermentation improves the physicochemical characteristics, bioactive compounds, and antioxidant activity of acerola (Malpighia emarginata D.C.) and guava (Psidium guajava L.) fruit processing by-products. *3 Biotech*. 2023;13(9):315. doi:10.1007/s13205-023-03738-1

174. Peng X, Yue Q, Chi Q, et al. Microbial Diversity and Flavor Regularity of Soy Milk Fermented Using Kombucha. *Foods*. 2023;12(4):4. doi:10.3390/foods12040884

175. Tu C, Tang S, Azi F, Hu W, Dong M. Use of kombucha consortium to transform soy whey into a novel functional beverage. *J Funct Foods*. 2019;52:81-89. doi:10.1016/j.jff.2018.10.024

176. Coelho RMD, Almeida AL de, Amaral RQG do, Mota RN da, Sousa PHM de. Kombucha: Review. *Int J Gastron Food Sci*. 2020;22:100272. doi:10.1016/j.ijgfs.2020.100272

177. Al-Mohammadi AR, Ismaiel AA, Ibrahim RA, Moustafa AH, Abou Zeid A, Enan G. Chemical Constitution and Antimicrobial Activity of Kombucha Fermented Beverage. *Molecules*. 2021;26(16):16. doi:10.3390/molecules26165026

178. Chakravorty S, Bhattacharya S, Chatzinotas A, Chakraborty W, Bhattacharya D, Gachhui R. Kombucha tea fermentation: Microbial and biochemical dynamics. *Int J Food Microbiol*. 2016;220:63-72. doi:10.1016/j.ijfoodmicro.2015.12.015

179. Harrison K, Curtin C. Microbial Composition of SCOBY Starter

Cultures Used by Commercial Kombucha Brewers in North America. *Microorganisms*. 2021;9(5):5. doi:10.3390/microorganisms9051060

180. Andreson M, Kazantseva J, Kuldjärv R, et al. Characterisation of chemical, microbial and sensory profiles of commercial kombuchas. *Int J Food Microbiol*. 2022;373:109715. doi:10.1016/j.ijfoodmicro.2022.109715

181. De Filippis F, Troise AD, Vitaglione P, Ercolini D. Different temperatures select distinctive acetic acid bacteria species and promotes organic acids production during Kombucha tea fermentation. *Food Microbiol*. 2018;73:11-16. doi:10.1016/j.fm.2018.01.008

182. Phan-Van T, Pham-Quang H, Do AD. Cascara kombucha potential as a functional food for fasting glucose regulation, cholesterol control, and liver function modulation: in vitro and in vivo study. *Int J Food Sci Technol*. 2024;59(11):8908-8916. doi:10.1111/ijfs.17477

183. Saimaiti A, Huang SY, Xiong RG, et al. Antioxidant Capacities and Polyphenol Contents of Kombucha Beverages Based on Vine Tea and Sweet Tea. *Antioxidants*. 2022;11(9):9. doi:10.3390/antiox11091655

184. Battikh H, Bakhrouf A, Ammar E. Antimicrobial effect of Kombucha analogues. *LWT - Food Sci Technol*. 2012;47(1):71-77. doi:10.1016/j.lwt.2011.12.033

185. Martínez-Leal J, Ponce-García N, Escalante-Aburto A. Recent Evidence of the Beneficial Effects Associated with Glucuronic Acid Contained in Kombucha Beverages. *Curr Nutr Rep*. 2020;9(3):163-170. doi:10.1007/s13668-020-00312-6

186. Mendelson C, Sparkes S, Merenstein DJ, et al. Kombucha tea as an anti-hyperglycemic agent in humans with diabetes – a randomized controlled pilot investigation. *Front Nutr*. 2023;10. doi:10.3389/fnut.2023.1190248

187. Costa MA de C, da Silva Duarte V, Fraiz GM, et al. Regular Consumption of Black Tea Kombucha Modulates the Gut Microbiota in Individuals with and without Obesity. *J Nutr*. 2025;155(5):1331-1349. doi:10.1016/j.tjnut.2024.12.013

188. Sannapaneni S, Philip S, Desai A, Mitchell J, Feldman M. Kombucha-Induced Massive Hepatic Necrosis: A Case Report and a Review of Literature. *Gastro Hep Adv*. 2023;2(2):196-198. doi:10.1016/j.gastha.2022.09.014

189. Yang J, Kornet R, Ntone E, et al. Plant protein aggregates induced by extraction and fractionation processes: Impact on techno-functional properties. *Food Hydrocoll*. 2024;155:110223. doi:10.1016/j.foodhyd.2024.110223

190. Schutyser MAI, van der Goot AJ. The potential of dry fractionation processes for sustainable plant protein production. *Trends Food Sci Technol*. 2011;22(4):154-164. doi:10.1016/j.tifs.2010.11.006

191. Preece KE, Hooshyar N, Zuidam NJ. Whole soybean protein extraction processes: A review. *Innov Food Sci Emerg Technol*. 2017;43:163-172. doi:10.1016/j.ifset.2017.07.024

192. Stone AK, Karalash A, Tyler RT, Warkentin TD, Nickerson MT. Functional attributes of pea protein isolates prepared using different extraction methods and cultivars. *Food Res Int*. 2015;76:31-38. doi:10.1016/j.foodres.2014.11.017

193. Manzanilla-Valdez ML, Ma Z, Mondor M, Hernández-Álvarez AJ. Decoding the Duality of Antinutrients: Assessing the Impact of Protein Extraction Methods on Plant-Based Protein Sources. *J Agric Food Chem*. 2024;72(22):12319-12339. doi:10.1021/acs.jafc.4c00380

194. Pedrosa MM, Varela A, Domínguez-Timón F, et al. Comparison of Bioactive Compounds Content and Techno-Functional Properties of Pea and Bean Flours and their Protein Isolates. *Plant Foods Hum Nutr*. 2020;75(4):642-650. doi:10.1007/s11130-020-00866-4

195. Kalaydzhiev H, Georgiev R, Ivanova P, et al. Enhanced Solubility of Rapeseed Meal Protein Isolates Prepared by Sequential Isoelectric Precipitation. *Foods*. 2020;9(6). doi:10.3390/foods9060703

196. Monteiro CA, Levy RB, Claro RM, Castro IRR de, Cannon G. A new classification of foods based on the extent and purpose of their processing. *Cad Saúde Pública*. 2010;26:2039-2049. doi:10.1590/S0102-311X2010001100005

197. Slimani N, Deharveng G, Southgate D a. T, et al. Contribution of highly industrially processed foods to the nutrient intakes and patterns of middle-aged populations in the European Prospective Investigation into Cancer and Nutrition study. *Eur J Clin Nutr*. 2009;63(4):S206-S225. doi:10.1038/ejcn.2009.82

198. Asfaw A. Does consumption of processed foods explain disparities in the body weight of individuals? The case of Guatemala. *Health Econ*. 2011;20(2):184-195. doi:10.1002/hec.1579

199. Poti JM, Mendez MA, Ng SW, Popkin BM. Is the degree of food processing and convenience linked with the nutritional quality of foods purchased by US households?23. *Am J Clin Nutr*. 2015;101(6):1251-1262. doi:10.3945/ajcn.114.100925

200. Eicher-Miller HA, Fulgoni VL, Keast DR. Contributions of Processed Foods to Dietary Intake in the US from 20032008: A Report of the Food and Nutrition Science Solutions Joint Task Force of the Academy of Nutrition and Dietetics, American Society for Nutrition, Institute of Food Technologists, and International Food Information Council, , 4. *J Nutr*. 2012;142(11):2065S-2072S. doi:10.3945/jn.112.164442

201. Deschasaux M, Huybrechts I, Julia C, et al. Association between nutritional profiles of foods underlying Nutri-Score front-of-pack labels and mortality: EPIC cohort study in 10 European countries. *BMJ*. 2020;370:m3173. doi:10.1136/bmj.m3173

202. Julia C, Hercberg S. Nutri-Score: Evidence of the effectiveness of the French front-of-pack nutrition label. *Ernähr Umsch*. 2017;(64(12)):158-165. doi:10.4455/eu.2017.048

203. Petrus RR, do Amaral Sobral PJ, Tadini CC, Gonçalves CB. The NOVA classification system: A critical perspective in food science.

Trends Food Sci Technol. 2021;116:603-608. doi:10.1016/j.tifs.2021.08.010

204. Sadler CR, Grassby T, Hart K, Raats M, Sokolović M, Timotijevic L. Processed food classification: Conceptualisation and challenges. *Trends Food Sci Technol*. 2021;112:149-162. doi:10.1016/j.tifs.2021.02.059

205. Juul F, Martinez-Steele E, Parekh N, Monteiro CA. The role of ultra-processed food in obesity. *Nat Rev Endocrinol*. Published online July 14, 2025:1-14. doi:10.1038/s41574-025-01143-7

206. González-Gil EM, Matta M, Berstein FM, et al. Associations between degree of food processing and all-cause and cause-specific mortality: a multicentre prospective cohort analysis in 9 European countries. *Lancet Reg Health – Eur*. 2025;50. doi:10.1016/j.lanepe.2024.101208

207. Cordova R, Viallon V, Fontvieille E, et al. Consumption of ultra-processed foods and risk of multimorbidity of cancer and cardiometabolic diseases: a multinational cohort study. *Lancet Reg Health - Eur*. Published online November 2023:100771. doi:10.1016/j.lanepe.2023.100771

208. Barbaresko J, Bröder J, Conrad J, Szczerba E, Lang A, Schlesinger S. Ultra-processed food consumption and human health: an umbrella review of systematic reviews with meta-analyses. *Crit Rev Food Sci Nutr*. 2025;65(11):1999-2007. doi:10.1080/10408398.2024.2317877

209. Visioli F, Del Rio D, Fogliano V, Marangoni F, Ricci C, Poli A. Ultra-processed foods and health: are we correctly interpreting the available evidence? *Eur J Clin Nutr*. 2025;79(3):177-180. doi:10.1038/s41430-024-01515-8

210. Srour B, Kordahi MC, Bonazzi E, Deschasaux-Tanguy M, Touvier M, Chassaing B. Ultra-processed foods and human health: from epidemiological evidence to mechanistic insights. *Lancet Gastroenterol Hepatol*. 2022;7(12):1128-1140. doi:10.1016/S2468-1253(22)00169-8

211. Monteiro CA. Nutrition and health. The issue is not food, nor nutrients, so much as processing. *Public Health Nutr*. 2009;12(5):729-731. doi:10.1017/S1368980009005291

212. Monteiro CA, Cannon G, Levy R, et al. NOVA. The star shines bright. *World Nutr*. 2016;7(1-3):1-3.

213. McGhie TK, Rowan DD. Metabolomics for measuring phytochemicals, and assessing human and animal responses to phytochemicals, in food science. *Mol Nutr Food Res*. 2012;56(1):147-158. doi:10.1002/mnfr.201100545

214. Pavagadhi S, Swarup S. Metabolomics for Evaluating Flavor-Associated Metabolites in Plant-Based Products. *Metabolites*. 2020;10(5):197. doi:10.3390/metabo10050197

215. Jacobs DM, van den Berg MA, Hall RD. Towards superior plant-based foods using metabolomics. *Curr Opin Biotechnol*. 2021;70:23-28. doi:10.1016/j.copbio.2020.08.010

216. Klåvus A, Kokla M, Noerman S, et al. "Notame": Workflow for Non-Targeted LC–MS Metabolic

Profiling. *Metabolites*. 2020;10(4). doi:10.3390/metabo10040135

217. Moco S, Bino RJ, Vorst O, et al. A Liquid Chromatography-Mass Spectrometry-Based Metabolome Database for Tomato. *Plant Physiol*. 2006;141(4):1205-1218. doi:10.1104/pp.106.078428

218. De Vos RC, Moco S, Lommen A, Keurentjes JJ, Bino RJ, Hall RD. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nat Protoc*. 2007;2(4):778-791. doi:10.1038/nprot.2007.95

219. Tolstikov VV, Fiehn O. Analysis of Highly Polar Compounds of Plant Origin: Combination of Hydrophilic Interaction Chromatography and Electrospray Ion Trap Mass Spectrometry. *Anal Biochem*. 2002;301(2):298-307. doi:10.1006/abio.2001.5513

220. Sumner LW, Amberg A, Barrett D, et al. Proposed minimum reporting standards for chemical analysis Chemical Analysis Working Group (CAWG) Metabolomics Standards Initiative (MSI). *Metabolomics Off J Metabolomic Soc*. 2007;3(3):211-221. doi:10.1007/s11306-007-0082-2

221. Tsugawa H, Cajka T, Kind T, et al. MS-DIAL: Data Independent MS/MS Deconvolution for Comprehensive Metabolome Analysis. *Nat Methods*. 2015;12(6):523-526. doi:10.1038/nmeth.3393

222. R Core Team R: A Language and Environment for Statistical Computing (R Foundation for Statistical Computing, 2013). doi:https://www.r-project.org/

223. Hernandez MS, Kim YHB, Woerner DR, Brooks JC, Legako JF. Untargeted Metabolomics Reveals Divergent Metabolomes between Three Plant-Based Meat Alternatives and Two Lean Levels of Ground Beef. *ACS Food Sci Technol*. 2025;5(4):1632-1644. doi:10.1021/acsfoodscitech.5c00041

224. Fan X, Liu X, Yan Y, et al. Widely targeted metabolomics analysis reveals the effect of soybean hull polysaccharides on nonvolatile components of plant-based yogurt and its metabolic pattern. *Int J Biol Macromol*. 2024;279:135144. doi:10.1016/j.ijbiomac.2024.135144

225. Lou J, Ou M, Teng Y, et al. Comparative metabolomics analysis of the fermented plant-based meat analogue of soybean proteins. *Food Biosci*. 2023;56:103143. doi:10.1016/j.fbio.2023.103143

226. Broadhurst D, Goodacre R, Reinke SN, et al. Guidelines and considerations for the use of system suitability and quality control samples in mass spectrometry assays applied in untargeted clinical metabolomic studies. *Metabolomics Off J Metabolomic Soc*. 2018;14(6):72. doi:10.1007/s11306-018-1367-3

227. Krusinski L, Maciel ICF, Vliet S van, et al. Measuring the Phytochemical Richness of Meat: Effects of Grass/Grain Finishing Systems and Grapeseed Extract Supplementation on the Fatty Acid and Phytochemical Content of Beef. *Foods*. 2023;12(19). doi:10.3390/foods12193547

228. Polanowska K, Grygier A, Kuligowski M, Rudzińska M, Nowak J. Effect of tempe fermentation by three different strains of Rhizopus oligosporus on nutritional characteristics of faba beans. *LWT*. 2020;122:109024. doi:10.1016/j.lwt.2020.109024

229. Fedde S, Wießner M, Hägele FA, Müller MJ, Bosy-Westphal A. Ultra-processed foods and plant-based alternatives impair nutritional quality of omnivorous and plant-forward dietary patterns in college students. *Sci Rep*. 2025;15(1):4233. doi:10.1038/s41598-025-88578-0

230. Pellegrini B, Strootman LX, Fryganas C, Martini D, Fogliano V. Home-made vs industry-made: Nutrient composition and content of potentially harmful compounds of different food products. *Curr Res Food Sci*. 2025;10:100958. doi:10.1016/j.crfs.2024.100958

231. Kahleova H, Himmelfarb J, Barnard ND. Not all ultra-processed foods are created equal: a review. *BMJ Nutr Prev Health*. Published online January 5, 2026. doi:10.1136/bmjnph-2025-001358

232. Qu S, Kwon SJ, Duan S, Lim YJ, Eom SH. Isoflavone Changes in Immature and Mature Soybeans by Thermal Processing. *Molecules*. 2021;26(24):7471. doi:10.3390/molecules26247471

233. Riedl KM, Lee JH, Renita M, St Martin SK, Schwartz SJ, Vodovotz Y. Isoflavone profiles, phenol content, and antioxidant activity of soybean seeds as influenced by cultivar and growing location in Ohio. *J Sci Food Agric*. 2007;87(7):1197-1206. doi:10.1002/jsfa.2795

234. Gasiński A, Mikulski D, Kłosowski G, Kawa-Rygielska J. Influence of malting procedure on the isoflavonoid content of soybeans. *Sci Rep*. 2024;14(1):7184. doi:10.1038/s41598-024-57914-1

235. Grün IU, Adhikari K, Li C, et al. Changes in the Profile of Genistein, Daidzein, and Their Conjugates during Thermal Processing of Tofu. *J Agric Food Chem*. 2001;49(6):2839-2843. doi:10.1021/jf010028+

236. Kao TH, Lu YF, Hsieh HC, Chen BH. Stability of isoflavone glucosides during processing of soymilk and tofu. *Food Res Int*. 2004;37(9):891-900. doi:10.1016/j.foodres.2004.05.007

237. Pontes CM, da Hora AM, Leal LP, et al. Impact of pre-treatments and processing methods on the nutritional, techno-functional, and bioactive properties of lupine (*Lupinus albus*) flour. *Food Chem*. 2025;493:146029. doi:10.1016/j.foodchem.2025.146029

238. Mesías M, Wagner M, George S, Morales FJ. Impact of conventional sterilization and ohmic heating on the amino acid profile in vegetable baby foods. *Innov Food Sci Emerg Technol*. 2016;34:24-28. doi:10.1016/j.ifset.2015.12.031

239. Yeung CY, Lee HC, Lin SP, Yang YC, Huang FY, Chuang CK. Negative effect of heat sterilization on the free amino acid concentrations in infant formula. *Eur J Clin Nutr*. 2006;60(1):136-141. doi:10.1038/sj.ejcn.1602279

240. Gao T, Chen J, Xu J, et al. Screening of a Novel Lactiplantibacillus plantarum MMB-05 and Lacticaseibacillus

casei Fermented Sandwich Seaweed Scraps: Chemical Composition, In Vitro Antioxidant, and Volatile Compounds Analysis by GC-IMS. *Foods*. 2022;11(18). doi:10.3390/foods11182875

241. Zaworska A, Frankiewicz A, Kasprowicz-Potocka M. The influence of narrow-leafed lupin seed fermentation on their chemical composition and ileal digestibility and microbiota in growing pigs. *Arch Anim Nutr*. 2017;71(4):285-296. doi:10.1080/1745039X.2017.1329130

242. Elango D, Rajendran K, Van der Laan L, et al. Raffinose Family Oligosaccharides: Friend or Foe for Human and Plant Health? *Front Plant Sci*. 2022;13. doi:10.3389/fpls.2022.829118

243. Kanwal F, Ren D, Kanwal W, Ding M, Su J, Shang X. The potential role of nondigestible Raffinose family oligosaccharides as prebiotics. *Glycobiology*. 2023;33(4):274-288. doi:10.1093/glycob/cwad015

244. Gardini F, Özogul Y, Suzzi G, Tabanelli G, Özogul F. Technological Factors Affecting Biogenic Amine Content in Foods: A Review. *Front Microbiol*. 2016;7. doi:10.3389/fmicb.2016.01218

245. Turna NS, Chung R, McIntyre L. A review of biogenic amines in fermented foods: Occurrence and health effects. *Heliyon*. 2024;10(2). doi:10.1016/j.heliyon.2024.e24501

246. Costa-Catala J, Tabanelli G, Díez-González A, Latorre-Moratalla ML, Vidal-Carou MC, Comas-Basté O. Managing bioactive amines in plant-based fermented products. *Food Control*. 2026;181:111749. doi:10.1016/j.foodcont.2025.111749

247. Vermeersch M, Mayr C, Maes A, et al. Microbiological survey of spontaneous vegetable fermentations: A food safety perspective. *Int J Food Microbiol*. 2026;446:111541. doi:10.1016/j.ijfoodmicro.2025.111541

248. Lee M, Song JH, Choi EJ, Yun YR, Lee KW, Chang JY. UPLC-QTOF-MS/MS and GC-MS Characterization of Phytochemicals in Vegetable Juice Fermented Using Lactic Acid Bacteria from Kimchi and Their Antioxidant Potential. *Antioxidants*. 2021;10(11):1761. doi:10.3390/antiox10111761

249. Zhou Q, Xie Z, Wu D, et al. The Effect of Indole-3-Lactic Acid from Lactiplantibacillus plantarum ZJ316 on Human Intestinal Microbiota In Vitro. *Foods*. 2022;11(20):3302. doi:10.3390/foods11203302

250. Valerio F, Lavermicocca P, Pascale M, Visconti A. Production of phenyllactic acid by lactic acid bacteria: an approach to the selection of strains contributing to food quality and preservation. *FEMS Microbiol Lett*. 2004;233(2):289-295. doi:10.1111/j.1574-6968.2004.tb09494.x

251. Wu H, Guang C, Zhang W, Mu W. Recent development of phenyllactic acid: physicochemical properties, biotechnological production strategies and applications. *Crit Rev Biotechnol*. 2023;43(2):293-308. doi:10.1080/07388551.2021.2010645

252. Qadi WSM, Mediani A, Benchoula K, Wong EH, Misnan NM, Sani NA. Characterization of Physicochemical, Biological, and Chemical Changes Associated with Coconut Milk Fermentation and Correlation Revealed by 1H NMR-Based Metabolomics. *Foods*. 2023;12(10):10. doi:10.3390/foods12101971

253. Maqbool MA, Aslam M, Akbar W, Iqbal Z. Biological importance of vitamins for human health: A review. 2017;02(03).

254. Feng ZY, Zhou JS, Qin ZH, Sheng R. Niacin Deficiency and Discovery of Vitamin B3 as a Precursor to Nicotinamide Coenzymes. In: Qin ZH, ed. *Biology of Nicotinamide Coenzymes: From Basic Science to Clinical Applications*. Springer Nature; 2025:11-16. doi:10.1007/978-981-97-9877-3_2

255. Kaźmierczak-Barańska J, Halczuk K, Karwowski BT. Thiamine (Vitamin B1)—An Essential Health Regulator. *Nutrients*. 2025;17(13). doi:10.3390/nu17132206

256. Hughes DE, Williamson DH. The deamidation of nicotinamide by bacteria. *Biochem J*. 1953;55(5):851-856. doi:10.1042/bj0550851

257. Rizzi F, Juan B, Espadaler-Mazo J, Capellas M, Huedo P. Lactiplantibacillus plantarum KABP051: Stability in Fruit Juices and Production of Bioactive Compounds During Their Fermentation. *Foods*. 2024;13(23):3851. doi:10.3390/foods13233851

258. Sneli EE, Strong FM, Peterson WH. Growth Factors for Bacteria: VIII. Pantothenic and Nicotinic Acids as Essential Growth Factors for Lactic and Propionic Acid Bacteria. *J Bacteriol*. 1939;38(3):293-308. doi:10.1128/jb.38.3.293-308.1939

259. Sierra I, Vidal-Valverde C. Vitamin B1 and B6 Retention in Milk after Continuous-Flow Microwave and Conventional Heating at High Temperatures. *J Food Prot*. 2001;64(6):890-894. doi:10.4315/0362-028X-64.6.890

260. Geraris Kartelias I, Karantonis HC, Giaouris E, Panagiotakopoulos I, Nasopoulou C. Kombucha Fermentation of Olympus Mountain Tea (Sideritis scardica) Sweetened with Thyme Honey: Physicochemical Analysis and Evaluation of Functional Properties. *Foods*. 2023;12(18):3496. doi:10.3390/foods12183496

APPENDIX: SUPPLEMENTARY MATERIALS

Supplementary Table 1. Annotated compounds in soy-based products.

Ionization	Mode	RT [min]	*m/z*	Adduct	Annotation	ID level	MS/MS spectra (relative intensity)
ESI+	HILIC	1.71	76.0765	[M+H]+	Trimethylamine N-oxide	1	76.074 (100)
ESI+	HILIC	6.35	86.0971	[M+H]+	Piperidine	2	86.096 (100)
ESI+	HILIC	4.23	104.0715	[M+H]+	gamma-Aminobutyric acid	1	87.044 (100), 86.06 (40), 104.07 (29), 69.033 (11), 88.047 (4)
ESI+	HILIC	1.6	104.1081	[M]+	Choline	2	104.107 (100), 60.081 (4)
ESI−	RP	2.62	109.0295	[M-H]-	Tannic acid	2	109.029 (100), 108.022 (9), 109.037 (5), 81.034 (4)
ESI+	HILIC	2.03	112.0516	[M+H]+	Cytosine	1	112.051 (100), 95.024 (91), 94.04 (35), 69.045 (9), 70.067 (8), 68.051 (6), 95.06 (5), 96.027 (5)
ESI+	RP	5.15	113.0599	[M+H]+	(2E,4E)-Hexa-2,4-dienoic acid	2	67.054 (100), 95.049 (51), 57.033 (24), 65.039 (10), 95.056 (9), 71.049 (9), 113.06 (7), 81.069 (6), 55.018 (6)
ESI+	HILIC	1.37	114.0666	[M+H]+	Creatinine	2	114.066 (100), 86.072 (8)
ESI+	RP	0.61	116.0715	[M+H]+	Proline	1	70.065 (100), 116.07 (15), 74.06 (11), 56.964 (6), 98.059 (5)
ESI−	RP	1.21	117.02	[M-H]-	Succinic acid	1	73.029 (100), 117.021 (29), 99.007 (18), 73.036 (6), 57.769 (4), 117.029 (4), 106.469 (4)
ESI−	RP	2.71	117.0558	[M-H]-	Hydroxyisovaleric acid	1	71.05 (100), 117.056 (57), 51.172 (8), 71.057 (6), 73.029 (5), 71.652 (5), 71.069 (4)
ESI+	HILIC	4.63	118.0865	[M+H]+	Valine	2	118.086 (100), 72.081 (46), 116.073 (10), 73.078 (9), 72.044 (5), 59.062 (4)
ESI+	HILIC	3.74	118.0874	[M+H]+	Betaine	2	118.086 (100)
ESI+	HILIC	5.76	120.0666	[M+H]+	Threonine	1	74.06 (100), 102.054 (49), 120.065 (49), 84.044 (11), 118.086 (8), 103.057 (4)
ESI−	RP	3.47	121.0295	[M-H]-	4-Hydroxybenzaldehyde	1	121.03 (100), 92.026 (65), 93.034 (11), 120.022 (10), 120.017 (7), 79.626 (4)
ESI−	RP	5.11	121.0296	[M-H]-	Benzoic acid	1	77.04 (100), 53.607 (9), 54.729 (8), 121.029 (7), 119.772 (7), 77.052 (4), 121.411 (4)
ESI+	RP	2.1	122.0972	[M+H]+	Phenylethylamine	2	105.07 (100), 103.054 (66), 77.039 (58), 79.054 (27), 68.032 (4), 105.093 (4), 103.062 (4), 105.079 (4)
ESI+	HILIC	0.74	123.0555	[M+H]+	Niacinamide	1	123.055 (100), 80.05 (17), 83.061 (7), 78.034 (4)
ESI+	HILIC	1.56	124.0404	[M+H]+	Nicotinic acid	2	124.04 (100), 124.088 (31), 80.051 (14), 78.036 (7)
ESI+	RP	1.98	127.0391	[M+H]+	1,2,3-Trihydroxybenzene	2	53.038 (100), 81.033 (50), 109.028 (29), 81.039 (13), 69.033 (11), 53.043 (9), 52.03 (4)
ESI+	RP	2.58	127.0394	[M+H]+	Maltol	2	127.039 (100), 53.038 (41), 109.028 (16), 81.032 (13), 87.007 (6), 127.06 (4), 127.064 (4)
ESI+	HILIC	4.94	130.0875	[M+H]+	Pipecolic acid	1	130.085 (100), 84.081 (47), 130.158 (4)
ESI−	RP	4.21	131.0717	[M-H]-	Leucic acid	2	85.066 (100), 131.071 (83), 92.553 (12), 85.073 (5)
ESI+	HILIC	6.16	131.1297	[M+H]+	Agmatine	1	72.082 (100), 114.104 (29), 131.129 (11), 97.078 (7), 73.085 (7), 60.058 (4)
ESI+	HILIC	5.59	132.0773	[M+H]+	Creatine	1	90.056 (100), 132.077 (83), 114.067 (5), 87.057 (4), 90.068 (4)
ESI−	HILIC	5.48	133.0171	[M-H]-	Malic acid	2	115.005 (100), 116.01 (14), 89.028 (11), 133.016 (10), 71.017 (9), 105.81 (8), 46.137 (8), 58.617 (8), 87.01 (7), 70.705 (7)
ESI+	HILIC	6.21	133.0618	[M+H]+	Asparagine	1	133.06 (100), 87.055 (74), 74.024 (60), 116.034 (37), 88.039 (21), 70.029 (4)
ESI+	HILIC	7.16	133.0983	[M+H]+	Ornithine	1	116.07 (100), 70.065 (44), 133.097 (22), 115.087 (18), 117.074 (5)
ESI+	HILIC	1.25	136.0629	[M+H]+	Adenine	2	92.024 (100), 119.034 (93), 65.014 (44), 77.014 (24), 82.04 (11), 67.029 (10), 83.043 (10), 94.039 (10), 136.064 (8), 66.034 (8)
ESI−	RP	3.02	137.0244	[M-H]-	4-Hydroxybenzoic acid	1	93.034 (100), 137.024 (31), 93.072 (6), 93.042 (6), 92.107 (4)
ESI−	RP	5.21	137.0244	[M-H]-	Salicylic acid	1	93.035 (100), 137.024 (22), 115.461 (10)
ESI+	HILIC	1.51	137.0465	[M+H]+	Hypoxanthine	2	137.045 (100), 121.065 (24), 110.035 (21), 119.035 (16), 136.061 (7), 94.04 (6), 93.069 (5), 103.055 (5)
ESI+	RP	2.12	138.0547	[M+H]+	p-Aminobenzoic acid	1	138.055 (100), 94.066 (49), 120.044 (40), 138.064 (13), 138.091 (12), 94.073 (11), 106.005 (9), 138.074 (6), 53.041 (6), 53.037 (5)
ESI+	RP	3.8	138.0551	[M+H]+	2-Aminobenzoic acid	1	120.044 (100), 92.049 (75), 65.037 (44), 60.056 (12), 65.042 (8), 92.056 (6), 120.151 (5), 65.045 (4)
ESI+	HILIC	4.14	138.0558	[M+H]+	Trigonelline	2	138.055 (100), 138.067 (15), 94.065 (14), 92.05 (11)
ESI+	RP	1.2	138.092	[M+H]+	Tyramine	2	77.038 (100), 121.064 (73), 91.054 (68), 103.054 (64), 93.069 (28), 81.033 (15), 92.049 (12), 119.048 (8), 103.058 (6), 103.735 (6)
ESI+	RP	0.98	140.0344	[M+H]+	6-Hydroxynicotinic acid	2	94.028 (100), 122.023 (87), 140.068 (23), 140.034 (21), 98.984 (11), 68.998 (11), 98.962 (10), 122.03 (6), 96.079 (5), 140.078 (5)
ESI+	HILIC	1.71	142.1236	[M+H]+	Pseudotropine	2	142.122 (100), 98.072 (15), 142.094 (8), 93.07 (7), 62.929 (6), 96.066 (6), 99.102 (5), 70.049 (5), 98.098 (5), 99.074 (5)
ESI+	RP	2.6	143.0339	[M+H]+	Kojic acid	2	143.034 (100), 143.042 (12), 69.033 (8)
ESI+	RP	1.93	144.0483	[M+H]+	5-(2-Hydroxyethyl)-4-methylthiazole	2	113.029 (100), 112.021 (83), 70.995 (55), 85.011 (31), 55.042 (24), 68.05 (24), 65.04 (24), 60.987 (15), 56.049 (15), 113.035 (12)
ESI+	HILIC	3.57	144.1023	[M+H]+	Proline betaine	1	144.1 (100)
ESI+	RP	4.67	146.0602	[M+H]+	1H-Indole-3-carboxaldehyde	2	118.065 (100), 146.06 (33), 118.071 (6), 117.056 (4), 145.969 (4)
ESI+	HILIC	3.89	146.0929	[M+H]+	4-Guanidinobutanoic acid	1	146.093 (100), 87.046 (49), 86.061 (46), 128.083 (13), 104.072 (11), 111.057 (8), 131.963 (5)
ESI−	RP	1.27	147.0299	[M-H]-	Citramalic acid	2	87.009 (100), 57.035 (93), 147.028 (61), 85.029 (52), 58.959 (26), 101.025 (22), 129.019 (21), 102.947 (20), 78.808 (12), 79.06 (11)
ESI+	HILIC	6.1	147.0775	[M+H]+	Glutamine	1	130.049 (100), 147.075 (40), 84.044 (30), 101.07 (8), 131.052 (6)
ESI+	HILIC	7.1	147.1139	[M+H]+	Lysine	1	130.086 (100), 84.081 (65), 147.112 (58), 129.102 (5), 131.089 (5), 85.084 (4)
ESI+	HILIC	6.33	148.0615	[M+H]+	Glutamic acid	2	148.06 (100), 84.045 (95), 130.049 (92), 102.055 (68), 131.052 (6)
ESI+	HILIC	4.34	150.0588	[M+H]+	Methionine	1	104.052 (100), 133.031 (83), 150.058 (71), 150.026 (19), 149.025 (16), 102.053 (13), 150.112 (11), 134.033 (11), 87.026 (9), 105.034 (8)
ESI−	RP	3.26	153.0193	[M-H-H2O]-	2-Pyrocatechuic acid	2	109.03 (100), 153.019 (98), 65.039 (22), 109.034 (18), 135.009 (18), 89.501 (14), 91.018 (12), 67.019 (8), 109.043 (6), 153.026 (6)

Ionization	Mode	RT [min]	*m/z*	Adduct	Annotation	ID level	MS/MS spectra (relative intensity)
ESI+	RP	2.27	154.0509	[M+H]+	3-Hydroxyanthranilic acid	2	136.04 (100), 108.044 (77), 80.05 (65), 136.046 (13), 53.038 (10), 67.02 (9), 67.055 (4)
ESI+	RP	9.09	159.117	[M+Na]+	Myrcene	2	131.085 (100), 159.117 (95), 117.07 (32), 91.054 (19), 130.078 (13), 116.063 (7), 159.126 (7), 144.093 (5), 159.133 (5), 159.083 (4)
ESI+	HILIC	2.28	160.1342	[M+H]+	5-Aminovaleric acid betaine	2	160.134 (100), 101.06 (32), 83.05 (10)
ESI−	RP	1.43	161.046	[M-H]-	3-Hydroxymethylglutaric acid	2	57.035 (100), 99.045 (71), 59.013 (28), 101.024 (25), 73.014 (10), 116.986 (10), 74.015 (8), 134.993 (8), 59.47 (7), 110.291 (6)
ESI+	RP	2.6	161.1076	[M+H]+	Tryptamine	2	115.054 (100), 117.056 (72), 91.053 (68), 90.046 (54), 65.037 (33), 77.039 (27), 101.04 (24), 90.053 (23), 126.047 (23), 89.04 (22)
ESI+	HILIC	4.23	162.1146	[M+H]+	Carnitine	2	162.111 (100), 103.038 (72), 85.028 (33), 102.091 (26)
ESI−	RP	4.25	163.0401	[M-H]-	p-Coumaric acid	1	119.05 (100), 163.039 (27), 119.057 (12), 115.392 (5)
ESI−	RP	4.46	165.056	[M-H]-	Phenyllactic acid	1	147.045 (100), 165.055 (53), 103.056 (38), 72.993 (29), 119.049 (18), 147.053 (11), 91.055 (11), 147.034 (4)
ESI+	RP	1.97	166.0884	[M+H]+	Phenylalanine	1	77.039 (100), 103.054 (54), 91.054 (26), 120.081 (12), 79.054 (12), 51.022 (5), 77.044 (5), 103.062 (4)
ESI+	RP	6.25	167.1067	[M+H]+	Perillic acid	2	84.96 (100), 149.096 (55), 121.065 (45), 121.101 (40), 109.065 (21), 91.055 (17), 125.986 (14), 107.05 (13), 81.069 (12), 125.06 (11)
ESI+	RP	2.07	169.0971	[M+H]+	Pyridoxamine	2	169.097 (100), 70.065 (18), 98.061 (13), 141.105 (11), 86.956 (6), 121.082 (5)
ESI−	HILIC	3.35	173.0105	[M-H]-	trans-Aconitic acid	2	85.031 (100), 129.021 (28), 111.01 (12), 86.035 (6)
ESI−	HILIC	5.49	173.0107	[M-H]-	cis-Aconitic acid	1	85.031 (100), 111.008 (99), 129.021 (81), 99.011 (69)
ESI−	RP	3.4	175.0612	[M-H]-	2-Isopropylmalic acid	2	175.061 (100), 115.04 (57), 85.066 (38), 130.966 (30), 113.061 (25), 131.071 (14), 175.069 (10), 85.073 (8), 129.056 (7), 86.977 (7)
ESI+	RP	3.75	175.0867	[M+H]+	1H-Indole-3-acetamide	2	77.039 (100), 130.066 (100), 103.054 (53), 102.047 (12), 103.058 (9), 72.938 (9), 51.023 (8), 84.959 (8), 128.048 (8), 89.038 (7)
ESI+	HILIC	6.96	175.1202	[M+H]+	Arginine	1	175.118 (100), 116.07 (7), 158.092 (4)
ESI+	HILIC	6.34	176.1034	[M+H]+	Citrulline	1	113.071 (100), 70.065 (74), 159.076 (46), 115.085 (10), 114.073 (9), 116.071 (8), 114.055 (7), 142.05 (5)
ESI+	RP	4.12	177.0546	[M+H-H2O]+	Herniarin	3	145.028 (100), 177.054 (54), 177.06 (19), 101.059 (13), 149.059 (11), 89.039 (10), 117.033 (8), 101.065 (7), 93.014 (7), 145.035 (6)
ESI−	RP	1.2	180.0668	[M-H]-	Tyrosine	2	180.066 (100), 163.039 (61), 93.034 (13), 119.049 (12), 163.049 (12), 106.042 (10), 164.042 (9), 72.009 (7), 163.696 (6), 59.015 (6)
ESI+	HILIC	2.04	180.1028	[M+H]+	Salsolinol	2	163.075 (100), 145.063 (86), 180.1 (46), 117.068 (45), 119.048 (22), 146.067 (20), 137.059 (18), 87.043 (16), 107.084 (14), 151.079 (14)
ESI−	RP	2.77	181.0508	[M-H]-	Hydroxyphenyllactic acid	1	181.051 (100), 163.041 (42), 135.045 (36), 119.051 (14), 68.995 (11), 86.986 (10), 180.138 (5), 136.921 (4), 181.06 (4), 93.035 (4)
ESI−	RP	5.68	187.0975	[M-H]-	Azelaic acid	2	125.097 (100), 187.097 (38), 123.081 (8), 169.086 (6), 127.379 (6), 125.103 (6), 187.107 (4), 143.106 (4), 187.109 (4)
ESI+	RP	2.18	189.1239	[M+H]+	Gly-Leu	2	86.096 (100), 132.102 (77), 143.118 (62), 87.1 (11), 86.102 (8), 189.123 (8), 103.962 (6), 132.108 (4), 143.124 (4)
ESI+	HILIC	6.75	189.1607	[M+H]+	N-6-Trimethyllysine	1	84.08 (100), 130.085 (42), 189.158 (18), 70.065 (10), 144.138 (7), 85.083 (5), 74.071 (5), 131.088 (5), 116.069 (4)
ESI+	HILIC	6.17	190.1191	[M+H]+	Homocitrulline	2	127.086 (100), 84.081 (47), 173.09 (23), 100.075 (11), 128.089 (9), 128.069 (9), 130.087 (6), 85.086 (5)
ESI+	HILIC	6.03	198.1251	[M+H]+	Histidine betaine	1	95.06 (100), 154.132 (28), 96.063 (6), 198.121 (4)
ESI+	RP	4.16	198.1292	[M+H]+	Dibenzylamine	2	91.054 (100), 198.128 (29), 181.102 (10), 106.064 (6), 91.06 (5), 91.076 (4)
ESI+	RP	2.62	205.0985	[M+H]+	Tryptophan	1	146.06 (100), 118.065 (54), 144.081 (28), 132.08 (16), 188.071 (13), 170.06 (13), 143.073 (9), 159.091 (7), 146.068 (5), 91.054 (5)
ESI+	RP	9.84	205.1955	[M+H]+	Prespatane	2	121.101 (100), 149.132 (37), 81.07 (31), 123.116 (29), 135.116 (24), 205.194 (24), 205.198 (24), 95.085 (21), 93.069 (11), 109.102 (10)
ESI−	RP	4.31	206.0822	[M-H]-	N-Acetylphenylalanine	2	206.083 (100), 164.071 (99), 91.055 (20), 147.046 (15), 171.03 (13), 135.324 (13), 117.556 (10), 205.996 (10), 206.108 (8), 50.893 (8)
ESI−	RP	3.66	210.0771	[M-H]-	Methoxytyrosine	2	124.04 (100), 210.077 (16), 106.029 (10), 163.096 (10), 124.048 (9), 94.03 (8), 196.084 (6), 96.004 (4)
ESI−	RP	2.37	218.1033	[M-H]-	Pantothenic acid	1	88.04 (100), 218.102 (33), 146.083 (19), 218.113 (4)
ESI+	HILIC	2.31	218.1394	[M+H]+	CAR 3:0	2	218.136 (100), 85.027 (13), 159.062 (8)
ESI+	RP	2.69	219.113	[M+H]+	Abrine	2	188.07 (100), 132.081 (41), 146.06 (17), 88.039 (13), 219.114 (6), 188.078 (4), 173.11 (4)
ESI+	RP	2.16	227.1032	[M+H]+	PyroGlu-Pro	2	116.07 (100), 227.103 (29), 181.097 (14), 70.065 (14), 84.044 (12), 209.093 (10), 116.074 (9), 73.029 (5), 152.032 (5), 227.111 (5)
ESI+	RP	7.52	228.1966	[M+H]+	Myristic acid	1	175.148 (100), 95.085 (70), 193.159 (58), 109.102 (58), 83.085 (52), 119.086 (40), 69.069 (39), 228.196 (38), 81.07 (33), 93.07 (31)
ESI+	RP	2.38	231.1713	[M+H]+	Ile-Val	2	86.096 (100), 231.17 (19), 69.07 (5), 86.102 (5), 97.065 (4)
ESI+	RP	2.86	231.1713	[M+H]+	Val-Leu	2	72.081 (100), 55.055 (10), 86.096 (7), 72.087 (6), 55.059 (4)
ESI+	HILIC	1.83	232.156	[M+H]+	CAR 4:0	2	85.029 (100), 232.155 (59), 173.081 (32), 86.033 (9)
ESI+	RP	2.21	233.1498	[M+H]+	Thr-Leu	2	74.06 (100), 86.096 (49), 56.049 (33), 84.045 (25), 113.108 (16), 74.064 (14), 151.026 (9), 113.116 (6), 74.069 (5), 58.065 (4)
ESI+	RP	8.87	235.17	[M+H]+	Curcumenol	2	133.1 (100), 119.086 (100), 105.069 (87), 121.101 (75), 95.085 (46), 97.101 (46), 93.07 (40), 91.055 (38), 81.07 (37), 189.163 (36)
ESI+	HILIC	7.09	241.1308	[M+H]+	Anserine	1	241.131 (100), 170.093 (35), 109.077 (21), 126.104 (13), 197.141 (10), 153.067 (10), 224.104 (7), 168.113 (6), 212.104 (5), 180.114 (5)
ESI+	HILIC	3.49	244.0946	[M+H]+	Cytidine	1	112.05 (100), 95.023 (27), 113.052 (9), 105.065 (7), 96.03 (7), 94.041 (6), 102.055 (5), 69.046 (4), 67.031 (4), 112.755 (4)
ESI+	RP	1.22	245.0768	[M+H]+	Uridine	2	113.034 (100), 57.034 (15), 113.042 (11), 152.07 (9), 86.032 (8), 96.008 (6), 55.017 (5), 113.174 (4), 73.03 (4), 83.966 (4)
ESI+	RP	2.77	247.1451	[M+H]+	Lenticin	2	146.061 (100), 188.07 (72), 60.08 (62), 118.065 (25), 144.082 (19), 142.066 (14), 91.054 (12), 144.044 (8), 146.067 (8), 160.076 (7)
ESI−	RP	3.69	250.0721	[M-H]-	Phenylacetylaspartic acid	2	132.03 (100), 135.047 (12), 250.077 (11), 88.039 (11), 232.065 (10), 250.07 (9), 90.8 (6), 91.056 (6), 132.056 (4), 91.06 (4)
ESI+	HILIC	1.11	252.1112	[M+H]+	Deoxyadenosine	2	136.061 (100), 252.107 (60), 252.08 (13), 250.176 (10), 137.061 (8), 250.057 (8), 117.089 (4)
ESI+	RP	9.63	252.2328	[M+H]+	(2E,4E)-N-(2-methylpropyl)dodeca-2,4-dienamide	2	252.232 (100), 179.143 (9), 252.244 (9), 57.07 (9), 81.033 (7), 196.174 (6), 97.102 (4), 252.269 (4), 140.107 (4)
ESI−	RP	5.95	253.052	[M-H]-	Daidzein	1	253.05 (100), 253.062 (8)

Ionization	Mode	RT [min]	*m/z*	Adduct	Annotation	ID level	MS/MS spectra (relative intensity)
ESI−	RP	2.29	255.0512	[M-H]-	Piscidic acid	2	165.056 (100), 255.051 (81), 179.035 (30), 193.05 (27), 72.992 (17), 111.009 (14), 107.049 (11), 209.045 (10), 58.006 (9), 149.064 (8)
ESI+	RP	2.96	261.145	[M+H]+	gamma-Glutamylleucine	2	132.103 (100), 86.097 (67), 261.144 (36), 198.112 (35), 244.118 (24), 130.05 (23), 244.122 (16), 132.11 (9), 170.117 (7), 123.118 (7)
ESI−	RP	5.98	263.1286	[M-H]-	Abscisic acid	2	153.092 (100), 219.138 (52), 204.115 (16), 204.119 (11), 152.083 (11), 153.1 (10), 111.045 (10), 201.128 (10), 263.132 (8), 219.148 (7)
ESI+	HILIC	3.86	265.1125	[M]+	Thiamine	1	122.071 (100), 265.111 (44), 144.047 (27), 123.074 (6)
ESI+	RP	3.35	265.1552	[M+H]+	Val-Phe	2	72.081 (100), 55.054 (51), 84.959 (14), 55.057 (12), 120.081 (9), 72.085 (5)
ESI+	RP	2.26	266.1409	[M]+	Caffeoylcholine	2	163.039 (100), 135.044 (98), 117.033 (71), 145.029 (47), 89.038 (45), 77.039 (13), 163.048 (11), 135.053 (7), 135.048 (6), 145.038 (6)
ESI+	HILIC	1.46	268.1053	[M+H]+	Adenosine	2	268.102 (100), 136.06 (25)
ESI−	RP	6.84	269.0455	[M-H]-	Apigenin	1	269.045 (100), 151.004 (20), 149.024 (16), 269.057 (10), 225.056 (8), 114.163 (7), 147.011 (7), 107.016 (6), 86.619 (6), 92.998 (6)
ESI−	RP	6.46	269.0469	[M-H]-	Genistein	1	269.045 (100), 269.082 (4)
ESI+	HILIC	2.64	269.0895	[M+H]+	Inosine	1	137.045 (100), 119.036 (6), 80.05 (5), 166.118 (5), 138.049 (5), 110.036 (5), 82.039 (4), 135.043 (4), 94.065 (4), 82.066 (4)
ESI−	RP	6.24	271.0612	[M-H]-	Naringenin	2	271.061 (100), 151.004 (24), 177.019 (23), 93.035 (21), 125.024 (7), 89.632 (4), 271.075 (4), 151.011 (4)
ESI+	RP	7.68	272.1289	[M+H]+	Piperyline	2	201.055 (100), 135.044 (44), 272.128 (32), 171.043 (23), 143.05 (18), 159.045 (13), 115.054 (12), 137.083 (8), 173.059 (7), 55.054 (7)
ESI+	RP	8.62	277.1806	[M+H]+	Shogaol	2	137.06 (100), 137.086 (4)
ESI+	RP	1.89	279.1012	[M+H]+	Glu-Met	2	150.058 (100), 279.101 (61), 133.031 (48), 56.049 (40), 104.052 (35), 130.05 (34), 168.068 (20), 262.075 (19), 261.091 (17), 84.044 (16)
ESI+	RP	10.22	280.264	[M+H]+	Linoleamide	1	280.263 (100), 263.237 (96), 245.227 (93), 109.101 (64), 175.149 (55), 95.086 (47), 57.069 (46), 100.076 (40), 181.159 (39), 114.091 (37)
ESI+	RP	2.14	282.1198	[M+H]+	Methyladenosine	1	150.078 (100), 57.034 (5), 150.087 (5)
ESI−	RP	8.07	283.0608	[M-H]-	Biochanin A	2	268.037 (100), 283.061 (25), 196.055 (11), 268.047 (9), 283.052 (8), 171.434 (6), 240.044 (5), 283.069 (5), 239.036 (5), 219.841 (5)
ESI−	RP	7.81	283.0614	[M-H]-	Glycitein isomer	1	268.037 (100), 283.059 (27), 132.021 (14), 268.049 (12), 239.035 (10), 267.031 (9), 240.04 (8), 172.161 (6), 283.069 (6), 241.135 (6)
ESI+	RP	1.39	284.0991	[M+H]+	Crotonoside	2	135.03 (100), 152.057 (78), 110.035 (37), 134.046 (6), 55.029 (5), 135.039 (5), 93.007 (4)
ESI−	RP	1.53	284.0996	[M+H]+	Guanosine	2	135.03 (100), 152.056 (30), 110.034 (14), 55.029 (7), 135.038 (7)
ESI−	RP	6.37	285.0402	[M-H]-	Luteolin	2	285.04 (100), 285.051 (12), 285.075 (4)
ESI+	RP	6.16	285.0759	[M+H]+	Glycitein	2	285.076 (100), 270.053 (20), 229.087 (10), 285.088 (8), 242.058 (6), 197.059 (6), 257.08 (5), 285.114 (4), 225.055 (4)
ESI+	RP	1.81	285.083	[M+H]+	Xanthosine	1	153.041 (100), 57.033 (57), 85.03 (34), 97.027 (25), 136.014 (24), 70.064 (21), 153.054 (17), 87.044 (15), 153.048 (15), 55.017 (13)
ESI+	RP	8.17	286.1472	[M+H]+	Piperine	1	115.054 (100), 143.049 (40), 135.044 (26), 171.044 (16), 69.07 (13), 201.055 (12), 173.06 (8), 77.038 (7), 103.053 (6), 159.045 (5)
ESI−	RP	5.65	287.0558	[M-H]-	Eriodictyol	2	151.003 (100), 287.054 (49), 135.045 (28), 185.711 (13), 221.551 (10), 287.062 (10), 185.716 (9), 52.228 (9), 151.011 (7), 161.022 (6)
ESI+	RP	8.08	288.1603	[M+H]+	Piperanine	2	288.16 (100), 135.044 (82), 86.096 (29), 138.091 (18), 161.06 (11), 98.097 (8), 153.114 (7), 288.17 (7), 124.112 (6), 69.069 (4)
ESI+	RP	3.35	295.1308	[M+H]+	Phe-Glu	2	120.081 (100), 84.044 (67), 103.054 (35), 56.049 (15), 91.055 (7), 77.038 (6), 107.05 (5), 79.054 (5), 186.092 (5), 120.087 (5)
ESI+	RP	2.85	298.0973	[M+H]+	5'-Methylthioadenosine	2	136.062 (100), 61.011 (7), 136.07 (4)
ESI−	RP	6.93	301.0715	[M-H]-	Stembin	2	165.02 (100), 301.072 (80), 135.045 (45), 129.1 (11), 176.284 (11), 278.547 (10), 109.343 (10), 301.085 (8), 161.024 (8), 77.438 (4)
ESI−	RP	3.46	305.0704	[M-H]-	Sulfojasmonate	2	305.07 (100), 305.08 (9), 59.014 (9), 96.959 (7), 258.931 (4)
ESI+	RP	8.13	306.207	[M+H]+	Capsaicin	2	137.06 (100), 122.036 (46), 94.042 (25), 66.046 (8), 79.054 (5), 69.07 (4), 81.07 (4), 137.086 (4)
ESI+	RP	8.52	308.2227	[M+H]+	Dihydrocapsaicin	2	137.06 (100), 122.036 (45), 94.042 (12), 79.054 (9), 57.07 (8), 66.046 (5), 69.07 (4), 137.068 (4)
ESI+	RP	7.92	309.1124	[M+H]+	Bisdemethoxycurcumin	2	119.049 (100), 91.054 (63), 147.045 (58), 77.039 (13), 131.05 (12), 154.078 (9), 65.04 (6), 119.074 (6), 147.416 (5), 103.053 (5)
ESI+	RP	3.09	310.1654	[M]+	Sinapine	2	251.091 (100), 310.164 (44), 169.097 (10), 251.105 (8), 310.177 (7), 273.966 (6), 101.062 (5), 251.119 (4)
ESI+	RP	2.13	311.1252	[M+H]+	Glu-Tyr	2	136.076 (100), 165.055 (82), 182.081 (32), 84.044 (23), 123.044 (19), 147.043 (19), 202.086 (16), 119.049 (16), 149.021 (13), 248.093 (13)
ESI−	RP	7.38	313.0713	[M-H]-	Cirsimaritin	2	313.071 (100), 298.049 (17), 283.024 (14), 313.083 (6), 181.941 (4)
ESI+	RP	5.66	314.1389	[M+H]+	Feruloyltyramine	2	177.054 (100), 121.065 (55), 145.028 (25), 177.063 (6), 314.136 (4), 121.071 (4)
ESI−	RP	2.15	315.072	[M-H]-	Dihydroxybenzoic acid hexoside	2	315.072 (100), 153.02 (19), 315.084 (11), 59.014 (8), 50.335 (8), 161.563 (7), 109.031 (7), 108.022 (6), 176.807 (5), 246.886 (5)
ESI+	RP	8.7	318.3008	[M+H]+	Phytosphingosine	2	318.301 (100), 60.044 (40), 300.291 (17), 270.28 (8), 282.279 (6), 179.105 (4), 318.34 (4)
ESI−	RP	1.21	328.0451	[M-H]-	3',5' cyclic AMP	2	134.048 (100), 328.045 (19), 78.662 (8), 134.056 (8), 104.282 (6), 96.858 (6), 328.053 (5)
ESI+	RP	8.76	331.1906	[M+H]+	Carnosol	2	285.184 (100), 267.174 (75), 215.108 (39), 289.142 (24), 331.191 (20), 201.057 (15), 243.138 (15), 69.069 (13), 263.127 (13), 225.129 (13)
ESI−	RP	7.85	343.0819	[M-H]-	Santin	2	343.081 (100), 328.057 (30), 313.035 (26), 343.059 (19), 270.013 (8), 317.038 (8), 343.096 (8), 243.128 (7), 313.047 (6), 313.041 (6)
ESI−	RP	1.3	344.0403	[M-H]-	3',5' cyclic GMP	2	150.042 (100), 344.04 (64), 150.05 (6), 52.954 (4)
ESI+	RP	9.67	344.2237	[M+H]+	Piperolein B	2	86.096 (100), 135.044 (89), 344.222 (60), 222.186 (20), 84.081 (15), 168.139 (12), 314.212 (10), 154.122 (9), 98.096 (8), 175.076 (8)
ESI−	RP	3.22	353.0892	[M-H]-	Chlorogenic Acid	1	191.056 (100), 353.087 (10)
ESI−	RP	2.06	355.0755	[M-H]-	Phenol esters	3	68.996 (100), 331.15 (9), 194.241 (7), 112.985 (7), 176.986 (5), 70.06 (4), 71.631 (4), 247.411 (4), 141.217 (4), 95.621 (4)
ESI−	RP	5.31	359.0776	[M-H]-	Rosmarinic acid	1	161.024 (100), 197.045 (26), 72.993 (22), 179.034 (22), 123.045 (9), 135.046 (7), 161.032 (7), 271.099 (4)
ESI−	RP	7.99	367.119	[M-H]-	Curcumin	1	134.037 (100), 149.061 (73), 158.036 (26), 173.059 (21), 175.041 (10), 149.055 (9), 250.694 (9), 202.027 (9), 217.047 (8), 144.709 (8)
ESI+	RP	6.73	369.1336	[M+H]+	Glycycoumarin	2	369.133 (100), 285.113 (95), 177.055 (74), 151.075 (49), 245.081 (46), 275.203 (31), 151.04 (28), 369.15 (27), 219.102 (27), 175.076 (23)

Ionization	Mode	RT [min]	*m/z*	Adduct	Annotation	ID level	MS/MS spectra (relative intensity)
ESI+	RP	4.18	377.1457	[M+H]+	Riboflavin	1	377.145 (100), 243.088 (97), 69.033 (23), 244.091 (14), 243.096 (14), 99.044 (13), 57.033 (10), 253.116 (10), 377.158 (7), 227.105 (6)
ESI+	RP	2.71	379.0635	[M+Na]+	Phenolic glucosides	3	379.063 (100), 233.027 (34), 147.046 (21), 147.042 (20), 169.026 (12), 379.08 (11), 379.072 (11), 169.032 (8), 148.046 (8), 333.835 (7)
ESI+	RP	3.41	395.1311	[M+Na]+	Syringin	2	395.131 (100), 233.079 (55), 232.07 (28), 185.041 (15), 395.141 (10), 364.113 (6), 395.147 (6), 364.119 (5), 233.089 (5), 349.085 (4)
ESI+	RP	4.21	417.1192	[M+H]+	Daidzein-hexoside	2	255.065 (100), 199.075 (17), 137.023 (10), 227.07 (9), 255.101 (4), 256.067 (4), 237.055 (4), 133.028 (4)
ESI+	RP	1.29	429.1367	[M+Na]+	Morroniside	2	429.136 (100), 429.149 (10), 267.086 (10), 152.058 (4), 429.182 (4)
ESI−	RP	5.41	431.0983	[M-H]-	Apigenin-glucoside	2	431.097 (100), 269.043 (15), 268.035 (8), 431.142 (4)
ESI−	RP	4.58	433.1135	[M-H]-	Naringenin-glucoside	2	433.115 (100), 271.061 (85), 271.072 (11), 145.029 (11), 433.128 (10), 153.018 (7), 433.202 (6), 151.004 (5), 313.055 (5), 169.013 (5)
ESI+	RP	4.73	433.1146	[M+H]+	Genistein-hexoside	1	271.06 (100), 433.11 (16), 271.07 (8), 271.095 (4)
ESI+	RP	3.62	433.1343	[M+H]+	Licoagroside B	2	127.039 (100), 271.082 (9), 85.028 (8), 433.13 (6), 433.136 (6), 127.047 (6), 145.049 (5), 433.167 (5), 163.06 (4)
ESI−	RP	5.37	445.0772	[M-H]-	Baicalein-glucuronide	2	269.045 (100), 113.024 (37), 445.084 (20), 445.077 (20), 175.024 (14), 351.993 (9), 99.009 (8), 87.158 (7), 445.097 (6), 89.46 (4)
ESI−	RP	2.5	447.0534	[M-H]-	Glucobrassicin	2	447.053 (100), 96.961 (16), 79.958 (11), 338.87 (9), 95.953 (9), 447.068 (9), 401.137 (7), 440.958 (7), 383.063 (6), 328.12 (6)
ESI−	RP	4.99	447.0931	[M-H]-	Luteolin-glucoside	1	447.092 (100), 285.042 (27), 285.037 (15), 447.106 (13), 447.137 (4)
ESI+	RP	4.37	447.129	[M+H]+	Glycitein-hexoside	2	285.076 (100), 285.088 (7), 285.112 (4)
ESI−	RP	3.67	449.1086	[M-H]-	Flavanomarein	2	449.108 (100), 287.056 (13), 449.123 (12), 259.061 (11), 269.044 (6), 259.065 (5), 329.064 (4), 431.098 (4), 449.154 (4)
ESI+	RP	5.48	459.1292	[M+H]+	Acetyldaidzein-hexoside	2	255.065 (100), 255.079 (5), 255.099 (4)
ESI−	RP	4.93	461.0721	[M-H]-	Kaempferol-glucuronide	2	285.041 (100), 461.067 (10), 87.008 (5), 133.029 (5), 425.2 (5), 117.019 (5), 286.044 (4), 87.014 (4)
ESI−	RP	5.39	463.0881	[M-H]-	Quercetin-hexoside	2	301.035 (100), 463.089 (48), 301.049 (5)
ESI+	RP	4.42	465.1031	[M+H]+	Quercetin-hexoside	2	303.049 (100), 85.028 (8), 153.018 (8), 304.055 (8), 303.059 (6), 111.007 (6), 257.044 (5), 165.017 (4), 201.056 (4)
ESI+	RP	9.72	468.3093	[M+H]+	LPC 14:0/0:0	2	104.107 (100), 184.074 (97), 86.097 (78), 125 (29), 60.081 (26), 166.064 (13), 57.07 (13), 229.213 (7), 184.082 (6), 163.017 (5)
ESI+	RP	6	475.1242	[M+H]+	Acetylgenistein-hexoside	2	271.06 (100), 272.064 (13), 271.07 (10), 271.074 (6), 475.176 (5)
ESI+	RP	5.62	489.1394	[M+H]+	Acetylglycitein-hexoside	2	285.075 (100), 489.14 (50), 430.808 (9), 286.58 (9), 399.809 (8), 412.85 (7), 285.11 (4)
ESI+	RP	4.64	503.1184	[M+H]+	Malonyldaidzein-hexoside isomer	2	255.065 (100), 255.073 (8), 199.074 (5), 256.065 (5), 153.07 (4), 255.101 (4), 149.023 (4)
ESI+	RP	5.09	503.1202	[M+H]+	Malonyldaidzein-hexoside	2	255.065 (100), 255.076 (6), 255.099 (4)
ESI+	RP	5.12	519.1136	[M+H]+	Malonylgenistein-hexoside isomer	2	271.06 (100), 519.11 (27), 519.117 (18), 271.069 (17)
ESI+	RP	5.97	519.1139	[M+H]+	Apigenin-glucoside isomer	2	271.06 (100), 153.018 (5), 272.063 (4)
ESI+	RP	5.54	519.1151	[M+H]+	Malonylgenistein-hexoside	2	271.06 (100), 271.071 (7), 272.064 (4)
ESI−	RP	4.96	521.1293	[M-H]-	Salviaflaside	2	161.024 (100), 135.045 (36), 179.035 (29), 323.078 (16), 72.993 (14), 133.031 (10), 117.035 (6), 197.048 (6), 160.016 (5), 297.995 (5)
ESI+	HILIC	3.2	533.1295	[M+H]+	Malonylglycitein-hexoside	2	285.077 (100), 286.08 (12)
ESI−	RP	5.33	563.1413	[M-H]-	Apigenin-apioglucoside	1	563.14 (100), 269.047 (84), 563.155 (12), 269.058 (8), 270.049 (5), 269.082 (4), 563.183 (4)
ESI−	RP	4.93	577.1196	[M-H]-	Flavonoid-hexoside C26H26O15 isomer	3	167.035 (100), 85.029 (48), 171.03 (42), 127.04 (40), 409.076 (36), 193.051 (35), 383.061 (31), 99.045 (24), 577.12 (23), 279.051 (23)
ESI−	RP	4.63	577.12	[M-H]-	Flavonoid-hexoside C26H26O15	3	193.05 (100), 167.034 (87), 517.098 (78), 235.062 (76), 85.029 (57), 577.116 (50), 127.04 (48), 171.03 (48), 215.02 (46), 577.124 (45)
ESI−	RP	5.51	593.1509	[M-H]-	Flavonoid-hexoside C27H30O15	3	593.148 (100), 299.056 (28), 593.169 (12), 387.451 (6), 388.854 (5), 185.045 (5), 299.068 (4), 244.707 (4), 593.178 (4), 527.495 (4)
ESI+	RP	4.04	595.1658	[M+H]+	Flavonoid-hexoside C27H30O15	3	595.166 (100), 577.152 (62), 379.086 (41), 595.635 (40), 457.112 (39), 475.102 (29), 457.121 (20), 463.099 (16), 595.182 (14), 578.196 (10)
ESI+	RP	6.14	595.2021	[M+H]+	2',6'-Dihydroxy-4-methoxychalcone-4'-O-neohesperidoside	2	287.091 (100), 433.147 (71), 449.144 (50), 415.137 (44), 329.102 (27), 397.128 (25), 431.134 (25), 559.182 (18), 263.056 (14), 395.115 (14)
ESI−	RP	5.55	607.1663	[M-H]-	Diosmetin-rutinoside	1	607.166 (100), 299.057 (38), 607.18 (11), 443.097 (9), 607.192 (7), 153.619 (7), 561.257 (7), 284.032 (6), 163.581 (5), 52.212 (4)
ESI−	RP	4.68	609.1455	[M-H]-	Cyanidin-glucopyranosyl-glucopyranoside	2	609.145 (100), 284.032 (17), 609.161 (14), 284.026 (10), 285.041 (8), 402.583 (6), 327.072 (6), 436.387 (5), 284.043 (5), 286.041 (5)
ESI−	RP	5.05	609.1456	[M-H]-	Quercetin-rutinoside	1	609.146 (100), 609.161 (14), 300.028 (11), 103.983 (5), 314.015 (5), 609.173 (4), 301.069 (4), 609.099 (4), 609.184 (4), 609.191 (4)
ESI−	RP	5.26	609.1827	[M-H]-	Hesperetin-rutinoside	1	609.183 (100), 609.199 (10), 301.07 (8), 609.356 (6), 609.136 (5), 609.235 (4), 65.235 (4), 403.102 (4)
ESI−	RP	4.48	623.1249	[M-H]-	Flavonoid-hexoside C27H28O17	3	623.125 (100), 623.145 (11), 285.041 (9), 318.911 (7), 252.329 (5), 311.561 (5), 565.253 (5), 383.326 (4), 458.944 (4), 623.184 (4)
ESI−	RP	4.4	625.1411	[M-H]-	Quercetin-dihexoside	2	463.087 (100), 625.14 (70), 301.036 (10), 301.03 (6), 464.09 (5), 272.315 (4), 463.132 (4)
ESI−	RP	8.86	911.5019	[M-H]-	Saponin C47H76O17	3	911.499 (100), 911.524 (26), 911.556 (6), 322.197 (5)
ESI−	RP	8.9	925.5158	[M-H]-	Saponin C48H78O17	3	925.514 (100), 925.538 (13), 50.513 (8), 340.112 (6), 597.383 (5), 833.39 (5), 809.015 (5), 112.986 (5), 206.034 (5), 925.574 (4)
ESI−	RP	3.32	933.2299	[M-H]-	Flavonoid-hexoside	3	771.179 (100), 609.142 (80), 771.166 (63), 716.169 (63), 866.832 (54), 891.619 (49), 188.801 (48), 664.827 (48), 870.749 (41), 112.154 (40)
ESI−	RP	9.17	939.4958	[M-H]-	Saponin C48H76O18	3	939.491 (100), 939.503 (64), 939.535 (4), 939.549 (4), 482.917 (4)
ESI+	RP	8.74	943.5278	[M+H]+	Soyasaponin Bb	2	943.527 (100), 797.467 (27), 781.474 (17), 617.405 (16), 943.548 (16), 441.376 (14), 441.369 (12), 423.361 (12), 599.389 (12), 635.417 (11)
ESI+	HILIC	4.87	943.5295	[M+H]+	Saponin C48H78O18	2	441.369 (100), 423.36 (61), 147.064 (29), 442.374 (26), 599.393 (20), 424.363 (18), 129.053 (16), 85.029 (12), 159.028 (12), 163.059 (12)

Supplementary Table 2. Annotated compounds in fermented lupin-based beverages.

Ionization	Mode	RT [min]	*m/z*	Adduct	Annotation	ID level	MS/MS spectra (relative intenstity)
ESI+	HILIC	6.171	86.0963	[M+H]+	Piperidine	2	86.096 (100), 69.069 (50), 86.103 (19), 69.082 (4)
ESI–	RP	0.854	89.0252	[M-H]-	Lactic acid	2	89.024 (100), 89.029 (6)
ESI+	HILIC	6.358	89.1072	[M+H]+	Putrescine	1	72.081 (100), 72.086 (4)
ESI+	HILIC	4.891	104.07	[M+H]+	Aminobutyric acid isomer 3	2	87.044 (100), 86.06 (32), 104.07 (19), 69.033 (14), 87.051 (10)
ESI+	HILIC	4.294	104.071	[M+H]+	Aminobutyric acid isomer 1	2	87.044 (100), 69.033 (31), 86.059 (24), 104.07 (16), 68.05 (7), 87.051 (6)
ESI+	HILIC	4.452	104.071	[M+H]+	Aminobutyric acid isomer 2	2	87.044 (100), 69.033 (35), 86.061 (31), 104.106 (12), 87.056 (5), 87.065 (5), 87.053 (5), 69.037 (4)
ESI+	HILIC	1.993	112.051	[M+H]+	Cytosine	1	95.024 (100), 112.05 (39), 69.045 (25), 94.041 (18), 52.017 (16), 68.014 (15), 94.047 (8), 95.045 (5), 68.02 (5)
ESI+	RP	1.199	113.035	[M+H]+	Uracil	2	113.035 (100), 70.028 (24), 96.008 (23), 59.061 (8), 70.032 (5)
ESI+	HILIC	4.774	116.071	[M+H]+	L-Proline	1	70.065 (100), 116.069 (14), 70.071 (6)
ESI–	RP	1.19	117.02	[M-H]-	Succinic acid	1	73.03 (100), 117.02 (25), 99.008 (11), 117.028 (4)
ESI–	RP	2.711	117.056	[M-H]-	2-Hydroxy-3-methylbutyric acid	1	117.056 (100), 71.05 (92), 116.929 (21), 117.063 (18), 112.985 (13), 112.993 (5), 71.056 (4), 80.197 (4), 108.858 (4)
ESI+	HILIC	5.717	120.066	[M+H]+	Allothreonine	1	56.049 (100), 74.06 (95), 102.055 (33), 120.064 (24), 57.033 (10), 74.78 (7), 74.238 (6), 102.063 (5), 102.304 (4), 74.078 (4)
ESI+	RP	2.142	122.097	[M+H]+	Phenylethylamine	1	105.07 (100), 122.053 (12), 122.096 (11), 79.055 (10)
ESI+	RP	0.927	123.055	[M+H]+	Niacinamide	2	53.038 (100), 78.033 (62), 80.049 (52), 52.017 (18), 80.287 (6), 53.055 (6), 53.045 (5)
ESI+	RP	0.887	124.039	[M+H]+	Nicotinic acid	2	53.038 (100), 78.034 (77), 52.017 (51), 80.049 (32), 51.022 (26), 79.041 (23), 52.031 (22), 53.382 (12), 51.027 (7), 53.053 (4)
ESI+	RP	1.035	130.05	[M+H]+	Pyroglutamic acid	2	84.044 (100), 130.051 (11), 56.049 (8), 84.081 (7), 84.051 (5), 102.055 (4)
ESI+	RP	0.628	130.086	[M+H]+	Pipecolic acid	1	84.081 (100), 130.086 (42), 84.088 (12), 70.066 (10)
ESI–	RP	4.207	131.072	[M-H]-	Leucic acid	2	85.066 (100), 131.071 (53), 85.073 (6), 113.06 (5), 50.697 (4), 92.663 (4)
ESI+	HILIC	3.91	132.102	[M+H]+	Leucine	1	86.097 (100), 132.103 (10), 86.1 (7), 86.624 (4)
ESI+	RP	1.366	132.103	[M+H]+	Isoleucine	1	86.096 (100), 69.07 (15), 86.103 (6)
ESI+	HILIC	6.162	133.061	[M+H]+	L-Asparagine	1	74.023 (100), 116.032 (26), 133.06 (24), 87.055 (19), 116.071 (8), 116.04 (7), 88.075 (4), 88.078 (4), 74.043 (4), 116.05 (4)
ESI+	HILIC	7.063	133.098	[M+H]+	Ornithine	1	70.065 (100), 116.071 (86), 133.097 (9), 116.136 (5), 70.072 (4)
ESI+	HILIC	6.667	134.045	[M+H]+	Aspartic acid	1	74.023 (100), 88.039 (33), 88.045 (10), 74.03 (8), 70.029 (6), 134.047 (4)
ESI+	HILIC	1.187	136.062	[M+H]+	Adenine	1	136.061 (100), 119.035 (65), 136.07 (10), 92.025 (8), 119.043 (6), 67.028 (5), 136.065 (4)
ESI+	RP	0.955	137.046	[M+H]+	Hypoxanthine	1	137.046 (100), 94.04 (43), 55.029 (23), 110.035 (22), 119.035 (19), 67.029 (12), 82.039 (12), 137.107 (10), 83.025 (6), 137.053 (6)
ESI+	HILIC	4.095	138.056	[M+H]+	Trigonelline	1	138.055 (100), 92.049 (60), 94.065 (59), 78.034 (27), 65.038 (16), 77.038 (12), 53.039 (10), 79.042 (9), 93.058 (7), 138.064 (7)
ESI+	HILIC	1.432	138.092	[M+H]+	Tyramine	2	121.065 (100), 91.054 (73), 77.038 (65), 103.054 (50), 93.07 (42), 93.077 (7), 121.072 (6), 77.044 (4), 112.474 (4), 121.088 (4)
ESI+	HILIC	0.753	139.05	[M+H]+	Urocanic acid	2	121.04 (100), 93.045 (7), 98.059 (7), 139.086 (6), 121.047 (6), 139.092 (4)
ESI+	HILIC	1.4	140.082	[M+H]+	4-Amino-5-hydroxymethyl-2-methylpyrimidine	2	140.082 (100), 81.045 (93), 122.072 (18), 99.055 (12), 99.061 (10), 140.34 (6), 140.11 (5), 81.062 (5), 140.432 (5), 98.134 (4)
ESI+	HILIC	5.7	142.097	[M+H]+	L-Histidinol	1	124.087 (100), 83.06 (28), 95.061 (27), 81.045 (25), 142.098 (25), 125.072 (16), 82.052 (10), 81.05 (5), 142.506 (4), 142.102 (4)
ESI+	HILIC	5.248	143.081	[M+H]+	Ectoine	2	143.082 (100), 101.071 (82), 97.077 (39), 68.049 (26), 101.078 (11), 97.08 (6), 68.054 (5), 143.439 (5), 101.094 (4), 143.096 (4)
ESI+	RP	1.655	144.065	[M+H-H2(	L-2-Amino-6-oxohexanoic acid	2	98.061 (100), 55.017 (58), 70.065 (49), 55.054 (27), 98.962 (21), 72.985 (11), 70.07 (6), 55.022 (4), 98.069 (4)
ESI+	HILIC	3.575	144.102	[M+H]+	(2R)-6-methylpiperidine-2-carboxylic acid	2	144.102 (100), 84.078 (7), 144.11 (7), 98.098 (7), 144.049 (5)
ESI–	RP	2.623	145.051	[M-H]-	Apidic acid	1	101.061 (100), 83.05 (100), 145.052 (34), 127.04 (31), 65.161 (19), 112.005 (15), 83.057 (14), 81.036 (14), 68.752 (11), 83.405 (7)
ESI–	HILIC	6.265	146.046	[M-H]-	L-Glutamic acid	1	128.035 (100), 102.056 (73), 146.046 (38), 128.043 (7), 136.956 (5), 128.06 (5), 146.057 (4)
ESI+	RP	4.647	146.06	[M+H]+	1H-Indole-4-carboxaldehyde	2	118.066 (100), 146.154 (25), 146.062 (19), 100.955 (15), 104.01 (6), 118.075 (5), 102.055 (5), 127.157 (4)
ESI+	HILIC	3.745	146.093	[M+H]+	4-Guanidinobutanoic acid	1	146.093 (100), 87.045 (41), 86.062 (28), 87.064 (23), 104.959 (17), 146.101 (9), 86.066 (9), 87.05 (6), 146.113 (5), 128.083 (5)
ESI+	RP	2.613	146.118	[M+H]+	N-Methylisoleucine	2	100.112 (100), 100.134 (4)
ESI+	HILIC	0.819	146.119	[M+H]+	Acetylcholine	2	87.044 (100), 146.118 (48), 60.081 (27), 146.123 (5), 87.005 (4)
ESI+	HILIC	7.533	146.165	[M+H]+	Spermidine	1	72.081 (100), 112.112 (43), 58.064 (13), 75.091 (12), 146.167 (12), 129.138 (10), 84.081 (8), 72.007 (7), 72.087 (5), 146.082 (4)
ESI+	HILIC	6.051	147.076	[M+H]+	L-Glutamine	1	130.05 (100), 84.044 (81), 147.075 (18), 130.058 (11), 86.061 (7), 56.049 (5), 101.07 (5)
ESI+	HILIC	6.995	147.113	[M+H]+	L-Lysine	1	84.08 (100), 56.049 (85), 55.053 (9), 69.15 (8), 84.102 (5), 67.054 (5), 56.054 (4)
ESI+	RP	0.876	150.058	[M+H]+	Methionine	2	56.049 (100), 61.01 (93), 104.054 (16), 87.026 (15), 102.055 (14), 61.016 (14), 67.113 (12), 105.036 (9), 70.066 (7), 74.023 (6)
ESI+	HILIC	2.085	150.078	[M+H]+	3-Methyladenine	2	150.078 (100), 109.051 (27), 123.066 (19), 57.044 (16), 94.041 (14), 150.085 (12), 133.052 (12), 108.056 (8), 92.025 (8), 135.055 (5)
ESI+	RP	0.786	152.057	[M+H]+	Guanine	2	135.03 (100), 152.056 (51), 110.034 (31), 80.024 (14), 61.011 (11), 135.038 (7), 134.045 (6), 107.036 (6), 152.066 (5), 61.016 (4)
ESI+	HILIC	6.694	156.077	[M+H]+	L-Histidine	1	110.071 (100), 83.06 (44), 93.045 (28), 95.06 (13), 82.052 (7), 81.045 (6), 110.079 (5), 56.049 (4)
ESI+	HILIC	4.102	160.037	[M-H]-	Indole-3-carboxylic acid	2	116.047 (100), 160.04 (12), 116.056 (5)
ESI–	RP	1.412	161.046	[M-H]-	3-Hydroxymethylglutaric acid	1	99.045 (100), 57.034 (80), 161.044 (37), 72.996 (17), 82.082 (15), 59.014 (13), 95.998 (12), 65.014 (11), 60.198 (9), 101.024 (9)
ESI–	RP	4.21	163.04	[M-H]-	4-Hydroxycinnamic acid	1	119.05 (100), 119.057 (9), 163.039 (8), 111.109 (5), 78.949 (4), 57.671 (4)

Ionization	Mode	RT [min]	*m/z*	Adduct	Annotation	ID level	MS/MS spectra (relative intenstity)
ESI−	RP	4.472	165.056	[M-H]-	Phenyllactic acid	1	147.045 (100), 165.056 (60), 103.055 (51), 119.05 (31), 72.993 (14), 147.054 (6), 123.323 (4), 165.064 (4)
ESI+	HILIC	1.29	166.072	[M+H]+	Methylguanine	2	166.073 (100), 166.098 (19), 166.083 (10), 123.425 (6), 149.07 (6), 125.072 (5), 123.077 (4)
ESI+	RP	1.988	166.087	[M+H]+	L-Phenylalanine	1	77.038 (100), 103.054 (67), 91.055 (16), 120.081 (11), 79.054 (11), 51.023 (8)
ESI+	HILIC	5.21	169.097	[M+H]+	Pyridoxamine	2	152.07 (100), 134.06 (40), 110.059 (26), 152.089 (5), 152.1 (4)
ESI−	RP	1.293	173.009	[M-H]-	Aconitic acid isomer	1	85.029 (100), 129.019 (8), 85.036 (5), 116.071 (4), 85.05 (4)
ESI−	RP	0.959	173.009	[M-H]-	Aconitic acid	2	85.03 (100), 129.02 (28), 111.008 (23), 85.035 (6)
ESI+	HILIC	6.836	175.12	[M+H]+	Arginine	1	175.119 (100), 70.065 (45), 116.071 (43), 60.055 (36), 158.093 (19), 130.098 (16), 175.128 (8), 157.107 (5), 70.07 (4), 60.061 (4)
ESI+	HILIC	0.767	175.123	[M+H]+	3-(Dimethylaminomethyl)indole	2	130.064 (100), 77.038 (98), 103.054 (90), 128.05 (33), 145.078 (26), 77.044 (13), 131.04 (12), 78.122 (5), 130.073 (5), 77.057 (5)
ESI+	HILIC	1.037	180.088	[M+H]+	6H-Purin-6-one, 2-(dimethylamino)-1,7-dihydro-	2	180.088 (100), 110.035 (43), 71.06 (17), 135.03 (8), 178.072 (8), 108.044 (6), 180.098 (5)
ESI−	RP	2.765	181.051	[M-H]-	Hydroxyphenyllactic acid	1	181.051 (100), 163.04 (61), 135.046 (53), 112.985 (39), 119.049 (37), 68.995 (14), 180.974 (11), 181.256 (9), 181.061 (8), 163.045 (8)
ESI+	RP	1.179	182.081	[M+H]+	L-Tyrosine	1	136.076 (100), 165.054 (47), 123.044 (26), 119.05 (15), 182.081 (6), 136.084 (6), 95.049 (4), 147.044 (4), 136.102 (4)
ESI+	HILIC	7.969	184.073	[M]+	Phosphocholine	1	184.073 (100), 60.081 (39), 184.111 (20), 86.096 (16), 142.097 (8), 184.171 (7), 184.086 (6), 184.106 (6), 184.101 (4)
ESI+	HILIC	6.057	188.176	[M+H]+	N8-Acetylspermidine	2	188.176 (100), 171.15 (46), 114.091 (26), 171.177 (5), 188.184 (4)
ESI+	HILIC	6.53	189.135	[M+H]+	Targinine	2	189.134 (100), 116.07 (45), 74.071 (29), 70.065 (22), 158.093 (15), 189.145 (8), 144.112 (7), 112.087 (6), 72.082 (6), 115.086 (5)
ESI+	HILIC	6.659	189.16	[M+H]+	N6,N6,N6-Trimethyl-L-lysine	1	189.159 (100), 130.086 (81), 84.08 (56), 60.08 (54), 189.134 (21), 74.071 (15), 84.087 (11), 189.166 (8), 60.085 (8), 70.065 (7)
ESI−	HILIC	7.989	191.02	[M-H]-	Citric acid	1	111.009 (100), 87.009 (31), 85.029 (30), 191.019 (23), 129.019 (11), 111.016 (5)
ESI−	HILIC	6.267	195.051	[M-H]-	Gluconic acid	2	195.051 (100), 75.009 (43), 129.02 (32), 99.008 (21), 87.009 (15), 159.031 (13), 89.024 (12), 90.589 (9), 80.917 (9), 85.029 (7)
ESI+	RP	3.535	195.088	[M+H]+	Caffeine	1	138.066 (100), 195.087 (59), 110.074 (13), 69.045 (13), 138.075 (8), 109.04 (8), 110.069 (7), 123.043 (6), 58.065 (4), 195.095 (4)
ESI+	HILIC	6.288	203.15	[M+H]+	Asymmetric dimethylarginine	2	70.065 (100), 116.071 (21), 88.085 (15), 88.078 (14), 158.131 (11), 203.152 (7), 70.07 (5), 57.046 (4)
ESI−	RP	4.498	204.067	[M-H]-	Indolelactic acid	1	204.067 (100), 158.061 (47), 186.057 (34), 130.065 (19), 72.994 (12), 128.05 (10), 186.053 (9), 131.98 (8), 96.96 (8), 158.071 (5)
ESI+	RP	2.612	205.098	[M+H]+	Tryptophan	1	146.06 (100), 118.065 (40), 144.08 (27), 188.071 (16), 159.092 (15), 132.081 (11), 130.065 (10), 143.073 (7), 170.06 (7), 146.069 (4)
ESI+	RP	2.061	219.134	[M+H]+	Seryleucine	2	132.102 (100), 60.045 (33), 173.126 (9), 201.125 (6), 219.141 (4)
ESI+	RP	2.738	220.119	[M+H]+	Zeatin	2	136.062 (100), 220.118 (80), 220.126 (23), 136.07 (16), 125.097 (15), 220.131 (13), 214.916 (11), 90.977 (9), 220.143 (5), 190.35 (4)
ESI+	RP	2.162	227.102	[M+H]+	PyroGlu-Pro	2	70.066 (100), 84.044 (54), 181.098 (39), 209.092 (23), 125.071 (21), 227.112 (20), 125.076 (18), 227.122 (10), 209.103 (9), 227.077 (9)
ESI+	RP	2.782	229.155	[M+H]+	Isoleucylproline	2	116.07 (100), 229.153 (55), 86.096 (43), 116.048 (8), 70.065 (7), 70.07 (4)
ESI+	RP	2.42	231.17	[M+H]+	Leu-Val	2	86.096 (100), 231.171 (26), 185.165 (20), 118.086 (20), 71.068 (13), 185.174 (11), 86.103 (5)
ESI+	RP	2.907	231.17	[M+H]+	Valylleucine	2	72.08 (100), 231.171 (13), 72.086 (9), 185.161 (9), 55.055 (4), 185.171 (4), 55.058 (4)
ESI+	RP	2.25	233.15	[M+H]+	Thr-Leu	2	74.06 (100), 86.096 (39), 101.072 (24), 74.066 (13), 58.065 (11), 84.044 (9), 187.143 (9), 132.102 (6), 58.07 (5), 74.074 (4)
ESI+	HILIC	1.208	235.181	[M+H]+	Angustifoline	2	193.134 (100), 235.18 (72), 112.076 (42), 114.091 (25), 217.169 (22), 136.112 (15), 150.091 (9), 235.187 (8), 67.053 (7), 193.141 (6)
ESI+	RP	0.769	244.093	[M+H]+	Cytidine	2	112.05 (100), 95.024 (39), 94.04 (18), 67.029 (16), 69.045 (10), 113.053 (10), 68.012 (4)
ESI+	RP	3.584	245.186	[M+H]+	Leu-Leu	2	86.096 (100), 86.102 (10), 245.186 (8), 245.098 (8), 166.999 (6), 132.101 (5), 86.117 (4)
ESI+	RP	1.733	247.181	[M+H]+	Sophocarpine	3	247.18 (100), 98.096 (9), 247.191 (5), 247.215 (4)
ESI+	HILIC	1.607	249.196	[M+H]+	Lupinine isomer	2	249.197 (100), 59.049 (10), 249.207 (8), 136.112 (6), 249.279 (5)
ESI+	HILIC	1.388	249.197	[M+H]+	Lupinine	2	249.197 (100), 249.207 (8), 124.087 (5), 249.231 (5), 114.091 (4)
ESI+	HILIC	1.064	252.109	[M+H]+	2'-Deoxyadenosine	1	136.062 (100), 252.111 (25), 117.053 (11), 136.071 (4)
ESI+	HILIC	6.14	258.11	[M+H]+	Glycerophosphocholine	1	104.107 (100), 86.096 (26), 184.073 (20), 125 (19), 60.08 (8), 104.114 (7), 166.061 (5)
ESI+	RP	2.291	261.145	[M+H]+	Glutamylleucine	2	243.133 (100), 197.129 (79), 132.102 (62), 86.096 (41), 84.045 (31), 225.125 (22), 243.144 (19), 261.144 (9), 132.125 (6), 197.14 (5)
ESI+	HILIC	3.577	265.112	[M]+	Thiamine	1	122.071 (100), 144.047 (18), 81.044 (10), 122.097 (4)
ESI+	HILIC	2.335	265.193	[M+H]+	Hydroxylupanine	2	265.191 (100), 152.107 (5), 265.2 (4), 247.18 (4), 265.204 (4)
ESI+	RP	1.465	268.104	[M+H]+	Adenosine	1	136.062 (100), 268.105 (39), 136.069 (9), 268.116 (6)
ESI−	RP	6.799	269.046	[M-H]-	Apigenin	2	269.047 (100), 151.004 (20), 117.035 (17), 225.055 (15), 149.024 (14), 107.014 (10), 201.057 (9), 226.058 (6), 105.034 (6), 269.082 (5)
ESI+	HILIC	7.121	276.167	[M+H]+	Arginylthreonine	2	175.118 (100), 60.055 (76), 116.07 (60), 70.065 (49), 112.087 (47), 276.167 (32), 259.141 (31), 199.108 (26), 258.118 (24), 115.087 (24)
ESI+	HILIC	6.761	277.103	[M+H]+	gamma-Glutamylglutamic acid	2	84.044 (100), 56.049 (20)
ESI+	RP	9.238	277.216	[M+H-H2(	9S-Hydroxy-10E,12Z,15Z-octadecatrienoic acid	2	277.216 (100), 121.101 (56), 135.117 (49), 93.069 (38), 95.085 (27), 69.069 (22), 91.054 (19), 177.164 (18), 213.163 (18), 149.134 (18)
ESI+	RP	9.489	279.233	[M+H-H2(	13-OxoODE	2	67.054 (100), 81.07 (45), 95.085 (16), 55.054 (15), 121.027 (11), 57.07 (10), 53.038 (10), 79.054 (9), 93.07 (8), 91.054 (7)
ESI+	RP	9.32	281.248	[M+H]+	Linoleic acid	2	281.247 (100), 263.238 (80), 245.226 (63), 167.143 (44), 81.069 (43), 207.173 (42), 161.133 (42), 69.069 (39), 83.085 (37), 123.118 (30)
ESI+	HILIC	4.291	282.12	[M+H]+	1-Methyladenosine	1	150.078 (100), 282.12 (56), 282.134 (5), 150.103 (4)
ESI+	RP	1.52	284.099	[M+H]+	Guanosine	1	152.056 (100), 284.097 (7), 152.067 (6)
ESI+	HILIC	7.158	284.135	[M+H]+	Histidylglutamine	2	284.135 (100), 110.072 (53), 266.124 (15), 249.1 (15), 147.077 (14), 156.077 (14), 110.079 (6), 164.931 (5), 284.151 (4), 84.043 (4)

Ionization	Mode	RT [min]	*m/z*	Adduct	Annotation	ID level	MS/MS spectra (relative intenstity)
ESI–	RP	5.703	285.041	[M+H]+	Rhein	2	285.04 (100), 241.051 (17), 285.05 (11), 285.169 (9), 285.057 (4)
ESI+	HILIC	7.094	285.12	[M+H]+	Histidylglutamic acid	2	110.071 (100), 83.06 (58), 93.045 (23), 81.045 (10), 56.049 (10), 66.033 (7), 82.052 (6), 110.078 (5), 148.085 (4), 120.056 (4)
ESI+	HILIC	2.337	287.173	[M+Na]+	Oxymatrine	3	287.172 (100), 287.184 (15), 287.211 (4)
ESI+	RP	7.846	293.211	[M+H]+	9-OxoOTrE	2	91.054 (100), 79.054 (70), 93.069 (64), 81.07 (63), 77.038 (58), 67.054 (42), 105.07 (41), 107.086 (30), 117.069 (28), 128.063 (26)
ESI–	RP	9.238	293.212	[M+H-H2(	13-HOTE	2	293.211 (100), 275.201 (14), 195.139 (7), 293.282 (5), 293.179 (4), 293.25 (4), 223.137 (4)
ESI+	RP	3.342	295.129	[M+H]+	Glutamylphenylalanine	2	166.086 (100), 120.081 (48), 295.129 (31), 232.097 (13), 278.102 (9), 166.094 (7), 186.092 (5), 120.089 (4)
ESI+	RP	8.152	295.227	[M+H]+	9-OxoODE	8	55.054 (100), 55.017 (87), 81.07 (75), 79.054 (33), 91.054 (30), 67.054 (29), 107.085 (28), 107.049 (26), 69.07 (24), 119.087 (21)
ESI+	RP	9.475	295.227	[M+H]+	13-Keto-9Z,11E-octadecadienoic acid	2	179.143 (100), 277.217 (78), 135.117 (35), 99.08 (27), 93.069 (18), 121.101 (18), 71.085 (14), 149.132 (13), 295.228 (12), 151.112 (11)
ESI–	RP	9.489	295.229	[M-H]1-	9-HODE	2	295.227 (100), 277.217 (41), 171.103 (22), 295.237 (11), 277.23 (4)
ESI+	RP	9.637	297.243	[M+H]+	12(13)-Epoxy-9Z-octadecenoic acid	2	279.232 (100), 99.081 (29), 297.244 (27), 261.221 (20), 71.085 (17), 147.117 (17), 243.21 (14), 153.127 (11), 95.085 (10), 165.127 (10)
ESI–	RP	6.919	299.056	[M-H]-	Chrysoeriol	2	284.033 (100), 299.056 (15), 256.038 (7), 284.044 (7)
ESI–	RP	9.942	299.26	[M-H]-	2-Hydroxystearic acid	2	299.259 (100), 141.128 (7), 253.256 (5), 299.268 (5), 299.275 (5), 287.629 (5), 299.15 (4), 297.243 (4)
ESI+	HILIC	7.389	303.178	[M+H]+	Arginylglutamine	2	70.065 (100), 84.044 (14), 60.056 (10), 112.087 (8), 98.061 (8), 130.05 (7)
ESI+	RP	1.211	307.083	[M+H]+	Epigallocatechin	2	84.045 (100), 130.05 (30), 231.043 (25), 243.042 (25), 162.022 (24), 257.077 (21), 244.044 (18), 235.022 (15), 110.049 (14), 177.035 (14)
ESI+	RP	0.877	308.091	[M+H]+	Glutathione	1	179.049 (100), 308.09 (58), 162.022 (40), 76.021 (38), 233.06 (16), 308.102 (10), 144.012 (9), 84.044 (9), 163.023 (7), 290.08 (6)
ESI+	RP	0.624	308.091	[M+H]+	Glutathione isomer	1	179.048 (100), 308.092 (49), 233.058 (37), 76.021 (30), 162.021 (25), 245.059 (15), 179.058 (13), 187.052 (11), 144.013 (10), 233.065 (9)
ESI+	HILIC	2.335	309.198	[M+Na]+	Hexadecanedioic acid	2	309.198 (100), 309.21 (11)
ESI+	RP	2.107	311.125	[M+H]+	gamma-Glutamyltyrosine	2	165.055 (100), 136.075 (92), 84.044 (49), 123.044 (29), 130.05 (27), 182.081 (26), 147.043 (25), 202.084 (11), 119.049 (10), 202.088 (9)
ESI–	RP	9.01	313.239	[M-H]-	12,13-DHOME	2	183.139 (100), 129.092 (60), 313.239 (45), 295.228 (27), 99.081 (21), 277.217 (20), 195.137 (13), 58.006 (7), 183.148 (6), 313.252 (5)
ESI+	RP	9.009	315.253	[M+H]+	9,10-DHOME	2	279.233 (100), 315.213 (34), 297.24 (28), 315.178 (19), 315.137 (19), 123.118 (18), 121.101 (15), 185.118 (12), 273.149 (12), 163.149 (12)
ESI+	RP	8.817	318.3	[M+H]+	Phytosphingosine	2	318.301 (100), 318.316 (19), 282.279 (10), 60.045 (8), 69.069 (4)
ESI+	HILIC	2.096	319.151	[M+H]+	Zearalenone	2	319.151 (100), 277.128 (21), 284.116 (8), 319.164 (7), 301.14 (5), 150.055 (4)
ESI+	RP	10.181	324.29	[M+H]+	Linoleoyl ethanolamide	2	62.06 (100), 67.054 (52), 55.054 (34), 79.054 (20), 81.07 (16), 95.087 (16), 91.054 (11), 93.069 (11), 95.084 (10), 105.069 (10)
ESI+	RP	0.615	325.113	[M+H-H2(	Melibiose	2	61.028 (100), 57.033 (29), 73.028 (26), 97.027 (16), 61.034 (13), 85.027 (12), 97.035 (4), 87.203 (4)
ESI+	RP	10.439	326.306	[M+H]+	Oleoylethanolamide	2	62.06 (100), 55.054 (35), 69.07 (24), 57.07 (24), 67.055 (14), 81.07 (13), 83.085 (12), 79.054 (11), 62.065 (11), 93.069 (9)
ESI–	RP	1.159	328.045	[M-H]-	Adenosine 2'3'-cyclic phosphate	1	134.047 (100), 134.054 (9), 92.025 (8), 107.036 (7), 103.308 (7), 82.674 (6), 109.055 (5)
ESI–	RP	7.763	329.234	[M-H]-	(Z)-5,8,11-trihydroxyoctadec-9-enoic acid	2	329.233 (100), 211.134 (79), 229.144 (60), 99.081 (11), 57.034 (10), 293.212 (10), 171.103 (9), 329.247 (8), 139.113 (8), 183.139 (8)
ESI–	HILIC	5.972	341.109	[M-H]-	Trehalose	1	341.109 (100), 89.024 (50), 179.055 (34), 59.014 (31), 113.023 (13), 161.044 (10), 101.026 (9), 59.019 (8), 107.037 (7), 131.644 (6)
ESI–	RP	1.231	344.04	[M-H]-	Cyclic GMP	1	150.042 (100), 344.04 (47), 344.051 (7), 150.052 (5)
ESI+	RP	0.825	348.071	[M+H]+	Adenosine monophosphate	2	136.062 (100), 348.07 (66), 348.083 (9), 292.086 (6), 136.071 (5), 136.086 (5), 348.334 (4)
ESI+	RP	9.121	353.269	[M+H]+	Monolinolenin	2	95.085 (100), 121.101 (85), 81.069 (83), 243.21 (61), 149.133 (58), 67.054 (53), 107.086 (50), 261.221 (49), 85.065 (48), 93.07 (47)
ESI+	RP	9.222	353.269	[M+H]+	Monolinolenin isomer	2	81.07 (100), 95.085 (73), 67.054 (70), 93.07 (66), 55.054 (49), 131.085 (49), 91.055 (34), 77.038 (27), 109.1 (26), 119.084 (25)
ESI+	RP	8.935	360.251	[M+H]+	Napelline	2	360.25 (100), 360.293 (4), 360.275 (4), 360.199 (4), 360.283 (4)
ESI–	RP	1.126	362.051	[M-H]-	Guanosine monophosphate	2	362.05 (100), 78.959 (36), 211.002 (22), 326.992 (11), 362.063 (10), 97.473 (9), 256.133 (9), 86.372 (8), 152.265 (6), 78.962 (5)
ESI+	HILIC	6.268	365.106	[M+Na]+	Palatinose	1	365.105 (100), 347.097 (24), 203.052 (18), 365.119 (14), 365.149 (4), 347.104 (4)
ESI+	HILIC	5.977	365.106	[M+Na]+	Sucrose	1	203.053 (100), 365.106 (88), 185.042 (45), 365.117 (6)
ESI+	HILIC	0.754	369.218	[M+H]+	Hirsutine	2	369.218 (100), 369.225 (16), 369.248 (4)
ESI+	RP	3.112	379.1	[M+Na]+	Gentiopicrin	2	379.099 (100), 379.112 (8), 217.049 (4), 185.042 (4)
ESI–	RP	4.698	431.098	[M-H]-	Vitexin	2	431.098 (100), 311.057 (43), 431.113 (11), 193.507 (10), 303.983 (8), 175.563 (8), 255.906 (7), 79.018 (6), 394.955 (6), 311.064 (5)
ESI–	RP	4.885	431.098	[M-H]-	Vitexin isomer	2	431.097 (100), 311.056 (19), 129.887 (12), 369.322 (11), 226.965 (11), 431.113 (11), 249.833 (10), 117.033 (8), 341.592 (8), 295.062 (6)
ESI+	RP	5.56	463.123	[M]+	Peonidin-3-glucoside	2	301.071 (100), 286.047 (38), 121.05 (9), 216.139 (9), 301.08 (5), 229.051 (5), 302.07 (5), 258.058 (4)
ESI+	HILIC	5.975	527.159	[M+Na]+	Melezitose	2	203.053 (100), 365.11 (65), 365.102 (57), 347.101 (33), 185.042 (13), 275.073 (12), 203.064 (10), 372.945 (10), 365.125 (9), 365.218 (5)
ESI+	HILIC	8.197	527.159	[M+Na]+	Raffinose	1	527.158 (100), 527.179 (7), 527.204 (5), 527.211 (5), 527.189 (4)
ESI–	RP	4.727	563.14	[M-H]-	Isovitexin-arabinose isomer	2	563.141 (100), 413.085 (7), 239.896 (6), 527.62 (5), 475.29 (5), 293.045 (5), 563.16 (4), 311.057 (4), 447.907 (4)
ESI+	RP	4.914	565.154	[M+H]+	Isovitexin-arabinose	2	313.071 (100), 283.059 (76), 415.1 (75), 367.081 (68), 397.088 (49), 337.071 (38), 433.11 (24), 379.076 (21), 433.116 (21), 69.033 (18)
ESI+	RP	5.273	565.155	[M+H]+	Apiin	2	271.06 (100), 565.153 (54), 433.112 (17), 271.071 (14), 565.167 (12), 271.052 (8), 458.919 (6), 433.128 (4), 271.075 (4), 271.097 (4)
ESI–	RP	3.697	593.15	[M-H]-	Flavonoid m/z 593	3	593.15 (100), 593.163 (14), 431.093 (11), 431.102 (9), 255.711 (5), 473.113 (5), 418.89 (5), 485.729 (5), 473.107 (4), 69.297 (4)
ESI–	RP	2.075	595.142	[M+H]+	Flavonoid m/z 595	3	595.142 (100), 595.159 (9), 542.799 (6), 460.089 (5), 514.791 (4), 595.194 (4)
ESI+	RP	3.978	595.166	[M+H]+	Apigenin-digalactoside	2	595.165 (100), 577.155 (57), 559.144 (25), 457.112 (22), 529.132 (14), 499.122 (11), 595.185 (9), 475.123 (9), 523.123 (6), 421.089 (4)

Ionization	Mode	RT [min]	*m/z*	Adduct	Annotation	ID level	MS/MS spectra (relative intenstity)
ESI–	RP	1.211	611.144	[M-H]-	Oxidized glutathione	2	611.145 (100), 306.078 (25), 611.162 (16), 508.621 (14), 338.047 (13), 344.041 (10), 272.09 (10), 310.078 (9), 314.205 (7), 392.755 (6)
ESI+	RP	3.984	617.148	[M+Na]+	Vicenin 2	2	617.146 (100), 617.162 (20), 497.107 (9), 599.138 (7), 497.097 (6)
ESI–	HILIC	8.009	665.214	[M-H]-	Stachyose	1	383.117 (100), 179.056 (59), 383.124 (50), 101.024 (45), 71.014 (40), 221.067 (26), 113.025 (24), 59.013 (24), 143.036 (23), 203.056 (21)
ESI+	HILIC	8.659	689.211	[M+Na]+	Stachyose isomer	1	689.212 (100), 365.104 (16), 527.158 (13), 509.144 (7), 347.101 (4)
ESI+	RP	3.932	749.19	[M+Na]+	Camelliaside B	2	749.19 (100), 749.21 (10), 749.247 (4)
ESI+	HILIC	0.722	758.569	[M+H]+	PC(16:0/18:2)	1	184.073 (100), 758.571 (98), 758.846 (13), 758.593 (11), 753.644 (5), 758.552 (5), 758.622 (5)
ESI+	HILIC	0.723	782.57	[M+H]+	PC m/z 782	3	782.567 (100), 184.074 (76), 782.585 (25), 723.489 (5), 782.602 (4)
ESI+	HILIC	0.721	784.585	[M+Na]+	1-Hexadecanoyl-2-octadecanoyl-sn-glycero-3-phosp	2	784.584 (100), 784.597 (63), 184.074 (28), 783.573 (17), 784.676 (14), 784.612 (8), 784.627 (5)

APPENDIX: ORIGINAL PUBLICATIONS

I. Reprinted from *Nature Food*, 2025, 6, 503–512. Springer Nature, an open access article published under the terms of the Creative Commons (CC-BY 4.0) license.

II. Accepted manuscript.

DOCTORAL THESES IN FOOD SCIENCES AT THE UNIVERSITY OF TURKU

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

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

73. **TOMMI KUMPULAINEN (2018)** The complexity of freshness and locality in a food consumption context
74. **XUEYING MA (2018)** Non-volatile bioactive and sensory compounds in berries and leaves of sea buckthorn (*Hippophaë rhamnoides*)
75. **ANU NUORA (2018)** Postprandial lipid metabolism resulting from heated beef, homogenized milk and interesterified palm oil.
76. **HEIKKI AISALA (2019)** Sensory properties and underlying chemistry of Finnish edible wild mushrooms.
77. **YE TIAN (2019)** Phenolic compounds from Finnish berry species to enhance food safety.
78. **MAIJA PAAKKI (2020)** The importance of natural colors in food for the visual attractiveness of everyday lunch.
79. **SHUXUN LIU (2020**) Fermentation with non-*Saccharomyces* yeasts as a novel biotechnology for berry wine production.
80. **MARIKA KALPIO (2020)** Strategies for analyzing the regio- and stereospecific structures of individual triacylglycerols in natural fats and oils.
81. **JOHANNA JOKIOJA (2020)** Postprandial effects and metabolism of acylated anthocyanins originating from purple potatoes.
82. **NIINA KELANNE (2021)** Novel bioprocessing for increasing consumption of Nordic berries.
83. **NIKO MARKKINEN (2021)** Bioprocessing of berry materials with malolactic fermentation.
84. **GABRIELE BELTRAME (2021)** Polysaccharides from Finnish fungal resources.
85. **SALLA LAITO (2022)** Bioactive compounds in oats and gut health.
86. **KANG CHEN (2022)** Multi-omics study on the effects of anthocyanin extracts from bilberries and purple potatoes on type 2 diabetes in Zucker diabetic fatty rats.
87. **WENJIA HE (2022)** Bioprocessing of alcoholic beverages from apples and pears: Effects of raw materials and processes on quality.
88. **TANJA KAKKO (2023)** Alternative approaches to improve the processing and quality of under-utilized fish.
89. **MIKAEL FABRITIUS (2023)** Mass spectrometric methodologies for analysis of triacylglycerol and phospholipid regioisomers in natural fats and oils.
90. **ELLA AITTA (2023)** Green technologies for the extraction of oil and protein from Baltic herring (*Clupea harengus membras*).
91. **AMRUTA KULKARNI (2023)** Effect of omega-3 deficiency and positional distribution of docosahexaenoic acid in triacylglycerols on tissue lipids in rats.
92. **LIZ A. GUTIÉRREZ QUEQUEZANA (2023)** Effect of cultivar, growth environment and developmental stage on phenolic compounds and ascorbic acid in potato tubers grown in Finland.
93. **MINNA ROTOLA-PUKKILA (2024)** The umami compounds in Nordic food raw materials and the effect of cooking.
94. **EIJA AHONEN (2024)** Impact of lipid structure and selected antioxidants on the oxidation of docosahexaenoic acid.
95. **MARINA FIDELIS (2024)** Valorization of underutilized biomass for biorefinery and food applications: Exploring the processing, plant material composition, bioactivity and fortified bread models.
96. **YUQING ZHANG (2025)** Structural analysis of triacylglycerols and bioavailability of docosahexaenoic acid from regio- and enantiopure triacylglycerols.
97. **HANY AHMED (2025)** Molecular insights to gut–brain communication: Metabolomics approach on lifestyle influences.
98. **JASMIN RAITA (2026)** Metabolomics-based approach to study the impact of food processing on plant-based protein-rich foods.

TURUN
YLIOPISTO
UNIVERSITY
OF TURKU