

LABORATORY of FOOD CHEMISTRY

Information on BSc and MSc thesis projects at the Laboratory of Food Chemistry





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A thesis project at the Laboratory of Food Chemistry

See study handbook for prerequisites for starting a thesis at food chemistry.

During your thesis project in Food Chemistry, you will study a (bio)chemical issue related to a specific food product, ingredient or biomass conversion. Through our research, we aim to:

- Provide detailed molecular compositions of raw materials, industrial byproducts and foods
- Understand the mechanisms behind chemical and enzymatic reactions in foods, in order to steer them to our benefit or reduce their negative impact
- Identify molecular patterns responsible for biological activity or reactivity of molecules
- Predict progression of (spoilage) reactions by modelling

By working on these aims, we contribute to important industrial and societal trends, such as the **transition to a <u>plant-based diet</u>**, the **development of <u>healthy ingredients</u>** and food products, and the <u>valorization of industrial byproducts</u>.

By joining us for a BSc or MSc thesis project, you will make a direct contribution to one (or more) of the above goals, and you will learn how to independently tackle future (industrial) challenges in an academic way. At the Laboratory of Food Chemistry we strive for <u>regular contact between students</u> <u>and supervisors</u> to maximize the learning curve during your thesis.

Bachelor thesis

During your BSc thesis you can design and conduct your own experiments in our laboratory within the framework of your supervisor's research. You will be guided by your supervisor to get your (first) hands on experience with our advanced equipment to analyze your samples.

Workshops

To boost your skills as a young researcher, we offer multiple workshops in e.g. data management, literature search, academic writing, presenting, and research design.

Thesis ring

You will work in a small thesis ring, in which you discuss parts of your report, to learn from other students. The thesis ring will come together multiple times during your thesis project.

Master thesis

Master thesis topics comprise laboratory work in which you design and conduct your own experiments. You get the opportunity to get trained to use our advanced equipment to generate data yourself. After processing and interpretation of these data you will condense them into a scientific report. Thesis rings will be held once every two weeks, to improve your writing skills.

Thesis topics at Food Chemistry

The main research topics of our Laboratory are (see also Table of Contents):

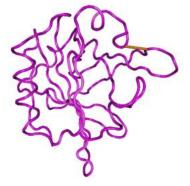
- Part 1: Food proteins
- Part 2: Plant bioactives
- Part 3: Phytochemicals
- Part 5: Food lipids
- Part 6: Flavor chemistry
- Part 7: Educational development
- Part 4: Biomass & Enzymology

On the following pages you can find the description of the research topics. For each topic several specific thesis projects are described. For many topics we describe the techniques that you may encounter (*HPAEC, GC, SPE, LC-MS, MALDI-TOF MS, etc.*). You may want to take your interest in specific techniques into account in the choice of topic. We are sure you will be able to find a topic that fits your interest. Many students have preceded you and enjoyed their thesis projects!

The staff of Food Chemistry

Part 1 Food proteins

Our aim is to gain knowledge of the effect of processing on the properties of proteins in raw materials, ingredients and foods, in relation to their functional and nutritional properties. Since proteins vary widely in their structure, their functional properties will diverge accordingly. Additionally, the functionality of proteins is influenced by the isolation procedure, chemical- or enzymatic reactions, and the composition and processing route of the food in which they are applied. Besides the use of proteins as food ingredient, we also focus on their breakdown by digestive enzymes. Knowledge of structure-function relationships of proteins in foods, and the interaction between proteins and other



food ingredients form the basis for the development of modern processes, new ingredients or higher quality products.

In the group there are four major research lines:

1) **Novel and current plant proteins (including microalgae):** Focused on isolation methods, functional challenges when replacing animal-based proteins, protein-phenol interactions, off-taste/off-colour formation and characterization of the obtained concentrates/isolates.

2) **Enzymatic hydrolysis of proteins**: To describe protein hydrolysis, a unique method has been developed to identify and quantify peptides at several stages of the hydrolysis process. This allows further development of concepts to characterize this process and relevant molecular properties of both proteases and substrate.

3) **Maillard reactions**: In order to understand the effects of Maillard reactions on ingredient functionality and protein digestibility using adequate methods that quantify the extent of modification.

4) **Foam- and emulsifying properties of proteins**: Several methods and concepts were developed to describe the properties of foams and emulsions.

Analytical methods used in the analysis of proteins and peptides are liquid-chromatography techniques as AEC, SEC, HILIC and advanced analytical techniques as LC-MS to analyse intact proteins and peptides. You can combine the information obtained with these techniques with protein conformation and protein functionality. We also have several pH-stat units to simulate in vitro digestion.

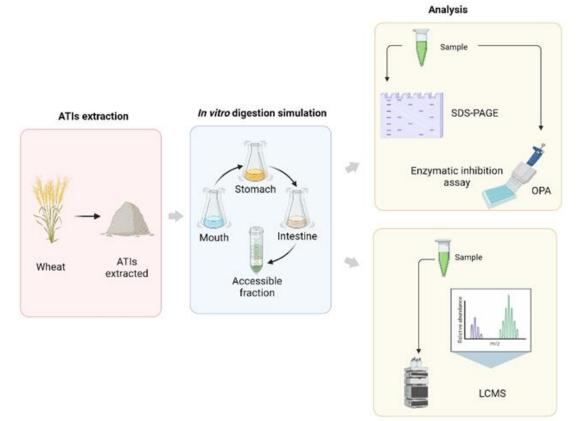
Topic 1.1 Irritable bowel syndrome and non-celiac wheat sensitivity: Focus on Amylase Trypsin Inhibitors stability throughout bread baking and digestion





Keywords:Supervisors:Dounia KrouchGijs VreekeHealth, allergies & intolerances, extraction,
digestionTopic suitable for: MSc

Amylase Trypsin Inhibitors (ATIs) are non-gluten proteins in wheat grains. ATIs are known for their highly stable structure due to the presence of 4-5 disulfide bridges, which are suspected to protect them from digestion enzymes and heat. Furthermore, by inhibiting trypsin and a-amylase, key digestive enzymes, ATIs actively contribute to the plant's defense against pests and pathogens. These proteins have recently been affiliated with diseases as irritable bowel syndrome and non-celiac wheat sensitivity. It is suspected that ATIs reach the gut intact and trigger intestinal inflammation in the gut immunological compartment. In this study, we aim to understand the stability of ATIs throughout the bread baking process and the digestion. To do so, first, the ATIs need to be extracted from dough and bread (think of normal bread, but also sourdough) and digested. Later, techniques, such as the OPA assay, SDS-PAGE, SEC and protease activity will be used to analyze the breakdown of the proteins. The results will help enhance understanding of ATIs' involvement in the related diseases.



MSc thesis project:

• Understand the stability of ATIs throughout bread baking and digestion.

Topic 1.2 Off-colour and off-flavour in plant protein isolates



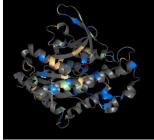


Keywords:	Supervisors:	Thore Diefenbach	Gijs Vreeke
Protein transition, functionality, enzyme characterization		Topic suita	ble for: Bsc & MSc

The plant protein market is growing fast and food products containing plant proteins are already widely available. Unlike protein isolates obtained from dairy or meat, plant protein isolates contain active enzymes, especially when they are extracted under mild processing conditions. These enzymes can be lipases, lipoxygenases or polyphenol oxidases which hydrolyse lipids (lipase), oxidize free fatty acids (lipoxygenase) and oxidise phenolic compounds (polyphenol oxidase). These activities have been found to be the cause for off-taste (e.g. in soy) and off-colour development (e.g. in sunflower) thereby leading to a lower applicability in food products.

In potato protein isolates the most relevant enzymes related to off-taste are patatin (lipase) and lipoxygenase isoforms. A main aim of this project is to better understand the enzymatic characteristics of these enzymes as well as their sensitivity to processing conditions (temperature, pH, ionic strength...) and other modifications (partial hydrolysis, glycation, crosslinking...). Also polyphenols such as chlorogenic acid might interact with the proteins and thereby influence their enzymatic activity and functionality. Eventually, this project aims to identify (ir)-reversible inactivation strategies of the enzymatic activities while maintaining functionality.





MSc. project(s):

 Investigate the enzymatic activities of patatin and lipoxygenases in potato protein isolates under different conditions and or modifications. Additionally, explore strategies to (ir)-reversibly inactivate these enzymes while keeping the functionality of the proteins.

BSc. project:

• Investigate by analysis of available data, the approaches used for other plant protein sources (soy, sunflower, lentil) to reduce the enzymatic activities of endogenous enzymes and propose ways to apply these approaches to potato proteins.

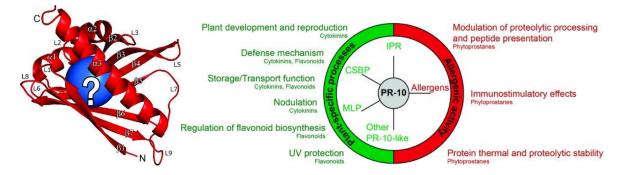
Techniques to be used: pH-stat, spectrophotometric assays, circular dichroism, LC-MS

Topic 1.3 Molecular characterisation of the cashew nut PR10 proteins



Keywords:	Supervisors:	Shanna Bastiaan-Net	Jean-Paul Vincken
Health, allergies & intolerances, microbiology, digestion		Торіс	c suitable for: MSc
5	Collaboration w	with WFBR and Avans Ho	aeschool Breda

Pathogenesis-related class 10 proteins (PR10) is a family of highly conserved small molecular weight plant proteins involved in the defence mechanism of plants. In addition, many of these PR10 proteins act as food allergens and can trigger Birch pollen allergy-related symptoms. All PR-10 proteins share a common structure characterized by a solvent-accessible hydrophobic cavity, which serves as a binding site for a myriad of small-molecule ligands, mostly phytohormones and flavonoids. Recently, we have identified several genes in cashew nut that transcribe PR10-like proteins, and we would like to characterise these proteins for their ligand-binding ability and their resemblance to Bet v 1.



We have cloned three different PR10 iso-allergens from cashew nut, which can be recombinantly expressed in E.coli. We suspect that the recombinant PR10 protein will be able to bind small-molecule ligands and are curious whether ligand binding changes their structure and/or stability. The aim of this Msc thesis is to purify the recombinantly produced proteins from E.coli and characterise them for several chemical aspects:

- Are the cashew PR10 proteins able to bind ligands?
- Does ligand binding influence their stability?

Foreseen activities:

- Stimulate E.coli clones to produce the PR10 protein (in Wageningen or Breda)
- Isolate and purify recombinant proteins for analysis (in Wageningen or Breda)
- Literature search for ligand candidates from cashew nut
- Evaluate ligand-binding capacity and how this influences stability (thermal and gastric digestion).

Techniques to be used: SDS PAGE, protein isolation, ANS assay, in vitro digestion

Topic 1.4Fish proteases and the effect of heating during feed production on protein
digestion

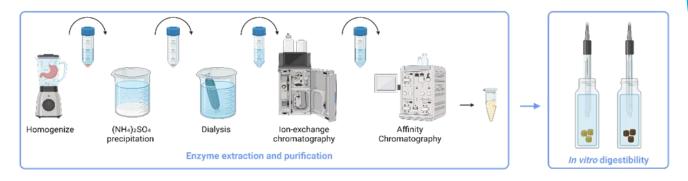




			-
Keywords:	Supervisors:	Abel Roijakkers	Gijs Vreeke
Enzyme characterization, digestion			Topic suitable for: MSc

In the production of fish feed, ingredients are processed at high temperatures to produce feed pellets. This can lead to inactivation of harmful compounds and thereby positively affect protein digestibility. However, heat treatments can also cause chemical reactions such as Maillard reactions (non-enzymatic browning) and protein cross-linking, which can lower protein digestibility. The current data available on the negative effect on protein digestibility are obtained from mammals (pigs). However, data on the effects in fish are limited. Therefore, this project aims to study the effect of feed processing on protein digestibility in fish.

First results from fish trials suggest, in contrast to mammals, limited or no effect of processing on the protein digestibility in fish. This might be attributed to differences in enzymes between fish and mammals. The role of proteases in *in vitro* digestibility trials can be evaluated using fish proteases. Fish often contain similar proteases as mammals: pepsin, trypsin and chymotrypsin, but as fish are cold-blooded animals, their enzymes often work at lower temperatures than those of mammals. In addition, fish proteases might have a different pH optimum, activity and specificity than mammal proteases. However, fish proteases are not commercially available and information on them is lacking. In this project, you are going to extract the enzymes from the fish tissue and compare the activity with the enzymes from cows and pigs.



MSc projects:

• Extraction, purification and characterisation of fish proteases for *in vitro* assessment of the effect of Maillard reaction and protein crosslinking on enzymatic protein digestion in fish.

Part 2 Plant Bioactives

Currently there is an increasing demand for natural, healthy and sustainable food ingredients. On top of that, antimicrobial resistance is a challenge not only in our healthcare, but in our foods and farm systems, and in our environment. Thus, there is a growing interest from science and industry to find potent 'clean label' natural antimicrobials to substitute traditional ones.

Plants produce an enormous variety of antimicrobial secondary metabolites (phytochemicals). Many of these phytochemical (e.g. phenolic compounds) can

be obtained through "by-products" from agroindustries, such as licorice root spent, soybean meal, wine pomace, etc. The valorization of such by-products to obtain natural antimicrobials represents also a great opportunity to optimize resource use and reduce food waste.

The Plant Bioactives group currently focusses on the search of promising plant antimicrobials. The aim is to (i) characterize the antimicrobial properties of phytochemicals against pathogenic and spoilage microorganisms; (ii) understand their (quantitative) structure-activity relationships; and (iii) elucidate their (molecular) mode of action.

1) Antimicrobial properties

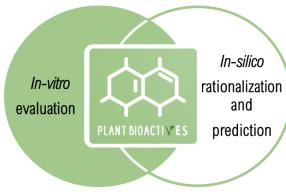
Phytochemicals have been shown to be effective antimicrobials against bacteria (cells and spores), fungi and viruses. An interesting strategy to control unwanted microorganisms is to design synergistic combinations of antimicrobials. For this, it is necessary to first characterize the different functionalities that different families of phytochemicals have (e.g. inhibition of specific proteins, damage of bacterial membrane, efflux inhibition, oxidative stress). Quantification of their activity and mapping their spectrum of activity is an essential part of this research. By using synergistic combinations of plant antimicrobials (i) the potency of the antimicrobial cocktail is increased; (ii) the dosage can be reduced, making the approach more feasible to be applied in e.g. foods; and (iii) the risk of persistent or resistant cells survival is significantly reduced.

2) Structure-activity relationships (SAR)

The activity of phytochemicals is strongly linked to their structure. Rather subtle structural differences can lead to a substantial change in the antimicrobial properties. Quantitative SAR analysis is a chemometric tool that allows to (i) predict activity for an efficient discovery and isolation of antimicrobial phytochemicals (e.g. limit the number of purification experiments or save purified compounds); (ii) speed up the design and optimization of lead antimicrobial compounds; (iii) provide insights into the molecular properties important for activity. A well-balanced approach using both a predictive assessment and *in-vitro* screening is necessary to guide this research.

3) Mode of action

To apply antimicrobials from plants in any setting, e.g. food or feed, their molecular mechanisms should be well-defined and validated. Elucidation of the molecular targets of antimicrobial phytochemicals using *in-vitro* assays is coupled with *in-silico* tools (molecular modelling). *In-vitro* assays include cell-based (fluorescence) assays, liposome model systems and MS-based (un)targeted analysis. *In-silico* tools include prediction of molecular properties, 3D pharmacophore modelling and molecular docking.



Topic 2.1Plant-derived antimicrobials to substitute traditional antibiotics:characterization of physicochemical properties of prenylated phenolics



Keywords:	Supervisors:		Carla Araya-Cloutier
Biofunctional food ingredients, modelling, microbiology		Topic su	iitable for: BSc & MSc

Antimicrobial resistance is one of the biggest threats to society, endangering human and animal health, food security and development. To combat antimicrobial resistance, novel and effective alternatives to traditional antimicrobial agents need to be developed. Prenylated phenolics are potent antimicrobial compounds produced by leguminous plants (*e.g.* soybean, peanut, or liquorice). To use prenylated phenolics as alternatives to traditional antimicrobials, their mechanism of antimicrobial action and safety should be assessed.

These natural compounds are hydrophobic due to the prenyl group (shown in red in **Figure 1**). This hydrophobic character influences many biological processes, for example interaction with antimicrobial targets (mechanism of antimicrobial action) and intestinal absorption *in-vivo* (safety). Hence, it is important to have valid measurements of hydrophobicity. This project aims to measure the hydrophobicity (LogD values) of antimicrobial prenylated phenolics. The goal is to develop and validate a screening method (*e.g.* miniaturized shake flask assay) to experimentally determine logD values of these natural compounds, to compare the experimental logD values with predicted logD values, and to link the hydrophobicity of prenylated phenolics to antimicrobial activity.

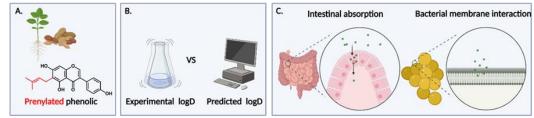


Figure 1A) Prenylated phenolic, **B)** Experimental determination and prediction of octanol-water partitioning (LogD values). **C)** Examples of biological processes influenced by LogD values.

Thesis project:

- *BSc project:* To develop and validate a method to experimentally define logD values of prenylated (iso)flavonoids.
- *MSc project:* To develop and validate a method to experimentally define logD values of prenylated (iso)flavonoids and to link to the distribution of prenylated (iso)flavonoids in antimicrobial activity tests.

Topic 2.2Food preservation using nature-inspired plant defence compounds

Keywords:	Supervisors:	Frenly Wehantouw	Carla Araya- Cloutier	Wouter de Bruijn
Biofunctional food ingredients, valorisation of by products, extraction, characterization, microbiology			Topic suitable	for: BSc & MSc

Is it possible to have a clean and green food preservative with highly potent antimicrobial properties? Novel natural antimicrobials are in high demand due to the increasing resistance of microorganisms against antimicrobials used in healthcare (predicted to result in 10 million deaths in 2050) but also in our food production systems. Furthermore, the food industry is looking for natural alternatives to synthetic food preservatives, due to consumer demands for more natural and plant-based food products. At the same time, the valorization of bioactive compounds from plant by-products (e.g. roots, stems, leaves) is of high interest to increase the sustainability of our current food production systems.

Plants contain secondary metabolites that have a wide variety of functions. One of those functions is as defence compounds. These defence compounds are produced in the plant in response to stress and can possess potent antimicrobial activity. Two promising classes of secondary metabolites are stilbenoids and (iso)flavonoids, which are produced, amongst others, by plants from the Fabaceae family, which includes commonly consumed legumes (e.g soybean). In a stressed plant, stilbenoids and (iso)flavonoids undergo two main types of modification to enhance their antimicrobial activity: prenylation and oxidative coupling. The latter contributes to the formation of dimers and larger oligomers (Figure 1). Prenylated phenolics can be found in several plant by-products, such as those from spent liquorice (*Glycyrrhiza* sp.) root, osage orange (*Maclura pomifera*) fruit, and peanut (*Arachis hypogaea*) skin or hull.

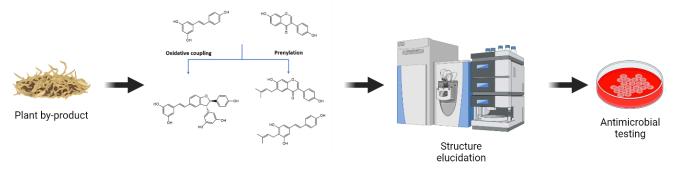


Figure 1. Schematic overview of the project

Possible projects:

The following projects are available to study antimicrobial properties of oligomeric (prenylated) phenolics:

• Investigate antimicrobial activity of dimeric prenylated phenolics from liquorice, osage orange, and peanut by-products (BSc/MSc thesis).



Part 3 Phytochemicals

Current trends in food and agriculture, such as the protein transition, are creating a growing interest in natural plant-based ingredients. As a result, phytochemicals (*phyto* meaning 'plant' in Ancient Greek) are becoming increasingly prevalent and important in food. The term 'phytochemicals' describes a bewildering number of small molecules from plants which can be divided into many distinct classes based on their biosynthetic origin,



including, but not limited to: (iso)flavonoids, stilbenoids , (hydroxy)cinnamic acids and their derivatives, and saponins. Some of these compounds are phenolics, i.e. they possess an aromatic ring substituted with at least one hydroxyl group (Figure 1). Phenolics are known to be reactive, as they can undergo oxidative coupling reactions which lead to dimerization or even further oligomerization.

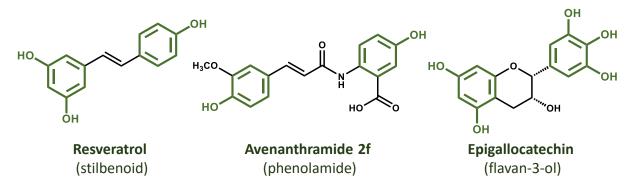


Figure 1. Structures of selected phytochemicals, phenolic groups are shown in bold and green.

Phytochemicals fulfil a wide variety of functions in plants, such as defence against biological threats or environmental stresses. Moreover, they can strongly affect food quality, despite the fact that they are present in much smaller quantities than carbohydrates, proteins, and lipids. In this respect, three main characteristics of phytochemicals are relevant in food technology: sensory properties (e.g. colour and taste), reactivity & interactions (e.g. oxidation, protein-phenolic interactions), and bioactivities (e.g. antimicrobial activity).

The aim of the FCH Phytochemicals Group is to (i) **characterize phytochemicals** from various plant materials using advanced analytical techniques; (ii) **monitor changes in phytochemical composition** during processing and storage of plant-based foods or ingredients; (iii) **modify phytochemicals** with chemical, enzymatic, or microbial approaches, in order to improve their properties; and (iv) **study interactions of phytochemicals** with proteins and micronutrients. Bioactive properties are determined in collaboration with the FCH Plant Bioactives Group.

Topic 3.1 Increasing the protein content in oat milk through protein-phenolic interactions

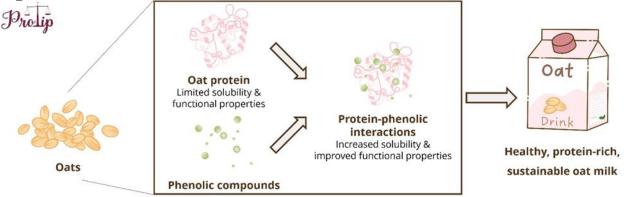




Keywords:	Supervisors:	Solange Ha	Wouter de Bruijn
Protein transition, characterization, functionality			Topic suitable for: MSc

Many of us like to consume healthy, sustainable foods with high-protein content. This has stimulated the food industry to find alternative **plant-based protein sources**. An alternative protein source is oats, due to their relatively high protein content of 15-20%. One of the main challenges in application of oat proteins is their low water solubility, resulting in **poor functional properties** at the food-relevant pH range of 4-7. You can also see this on oat milk packages because **commercially available oat milks have a low protein content** of approximately 0.3-1% proteins and a pH between 6.6 and 7.2. Previous research has shown that it is possible to increase protein solubility by forming interactions between **proteins and phenolic compounds**. Phenolic compounds are known to interact non-covalently and covalently with proteins. Various parameters, in particular the pH, can affect these interactions. However, most of the information on protein-phenolic interactions is based on studies with simple phenolics and soluble dairy proteins, rather than oat phenolics and oat proteins.

The aim of this project, with the name ProTip, is to increase the protein solubility of oat by modifying the protein with phenolic compounds from oat. Protein-phenolic interactions will be induced under varying conditions to investigate the effect on protein properties. Eventually the aim would be to **enable the production of sustainable and attractive oat-based foods and ingredients**.



Possible projects:

Modification of oat protein with various phenolic compounds, and

- Characterizing the modified protein and phenolic compounds using advanced analytical methods (e.g., LC-MS).
- Investigate the effect on protein functionality, in particular the solubility, using a model protein-phenolic system.

Topic 3.2 More than a passenger princess; unlocking the antioxidant potential of co-passengers of plant proteins in emulsions

Supervisors:





Wouter de Bruijn & Marie Hennebelle

Protein transition, extraction, characterization, functionality

Kevwords:

Topic suitable for: BSc & MSc

Stability of food products during storage is a key determinant of their quality and is essential in minimising food waste. Protein isolates or concentrates from legumes (e.g. soy, pea, faba bean) are used as plantderived ingredients for physical stabilization of emulsions. Interestingly, these plant protein ingredients also contribute to chemical stabilization of emulsions, by inhibiting lipid oxidation and thereby preventing rancidity. However, the compounds and mechanisms responsible for this protective effect are yet unknown. The current hypothesis is that non-protein constituents that are present in protein ingredients (called copassengers), such as phytochemicals and polar lipids, are primarily responsible. The main aim of this study is to identify these compounds in order to understand the underlying protective mechanisms. To this end, we will use extraction and fractionation approaches, combined with state-of-the-art liquid chromatography (LC), gas chromatography (GC), high resolution mass spectrometry (HRMS), and ion mobility spectrometry (IMS).

The outcomes from this study will provide (i) insights in the protective mechanisms of plant protein ingredients against oxidation in emulsions, (ii) leads for novel plant-derived antioxidants, and (iii) guidance for (milder) processing to obtain ingredients with optimized functionality.

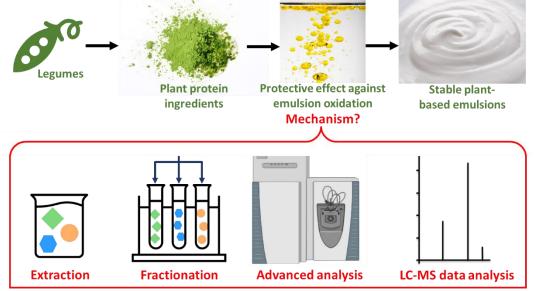


Figure 2: Overview of this study. The main activities for thesis projects are show in red.

Example BSc project:

Make an overview of literature on the antioxidative effect of plant protein ingredients, as well as performing extraction of plant protein ingredients and analysing the composition based on LC-MS data. Possibly preparing emulsions and performing a shelf-life analysis to determine the antioxidative effect.

Example MSc project:

Develop extraction, fractionation, and analytical approaches to characterize non-protein compounds that potentially contribute to the antioxidative effect of plant protein ingredients, such as phytochemicals and polar lipids. Possibly preparing emulsions and performing a shelf-life analysis to determine the antioxidative effect.

Part 4 Biomass & Enzymology

'Biomass and Enzymology' studies the changes in carbohydrates and of lignin during plant biomass conversion processes, and in fungal biomass. These processes are not only limited to the more well-known biorefinery's existing to produce food, fuels, and value-added chemicals from biomass, but also relating projects e.g. biomass composting for mushroom growth, or feed digestibility (animal nutrition) are part of this theme. Understanding of enzymatic routes to degrade the plant carbohydrates and lignin is a major topic within this research.

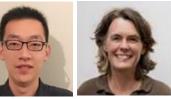
The aim of this research is to monitor changes in lignin, plant carbohydrates and fungal cell wall carbohydrates, such as chitin, during pretreatment, fungal growth, enzymatic processes, and during animal digestion. To upgrade said side-streams, or during fungal growth and digestion, various types of enzymes play key roles. Thus, we also take up the challenge to understand mode-of-action of hydrolytic and oxidative carbohydrate and lignin degrading/modifying enzymes and their effect on the chemical fine structure of natural substrates. A better understanding of the cell wall architecture's network will provide a better understanding of how to influence changes in biomass architecture and it's enzymatic (biological) degradation.

For the lignin analysis, we have set-up a new method via pyrolysis-GC-MS, making use of a mildly extracted ¹³C-lignin isolate from wheat straw as internal standard. This method allows us to specifically quantify residual lignin content *in situ*, while simultaneously providing structural insights. Further lignin characterisation via NMR (HSQC) has been set up. We now strive to set-up a similar method for chitin quantification. Carbohydrate analysis is applied in situ or/and after extraction polysaccharides. Examples of analysis are carbohydrate content and composition, the sugar linkage composition, the type and amount of substituents on the carbohydrates present. Various chromatographic and mass spectrometric techniques are available.

In carbohydrate biochemistry, structural composition studies are often closely related with the study of commercial and single-activity (hemi-)cellulases or esterases (enzymes). Currently, oxidative enzymes, such as the new lytic polysaccharide monooxygenases, are studied, in addition to polysaccharide lyases.

Topic 4.1 Discovery and characterisation of pectin-active enzymes

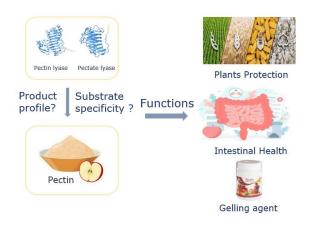




Keywords:	Supervisors:	Nan Zhang	Peicheng Sun	Mirjam Kabel
Enzymes, purification, characterization				
			Topic suitab	le for: BSc & MSc

Are you interested in enzyme discovery? Do novel pectin active lyases make you curious? Are you interested in how such enzymes can contribute to food?

Pectin-active enzymes are widely used in the food industry: they for example enhance the extraction and clarification of fruit juices, they improve the texture and consistency of jams and jellies, they facilitate the production of wine and beer by reducing haze, and they activity leads to the formation of (prebiotic) oligosaccharides. Albeit their large portfolio of applications, only few pectin-active enzymes are characterised, and in use.



The nowadays large availability of fungal (and bacterial) genomes, has immensely increased the number of *candidate* pectin-active enzymes. Here, enzyme discovery and characterisation will contribute to understand the natural portfolio of pectin-active enzyme properties, such as its substrate specificity, stability, temperature, and pH preferences.

In particular, the detailed understanding of substrate specificity is lacking behind. We do know that many *candidate* pectin active lyases cluster together in families based on protein sequence similarity, but we do not yet

understand variations in their substrate specificity, and product profiles.

In this project, students will be involved in the process of the production and purification of novel pectin-active enzymes and learn how to determine their catalytic performance, and substrate specificity and product profiles. During this project, you will be exposed to protein production and purification techniques and methods of chemical analysis, experiencing the fascination of enzymology.

MSc/BSc projects:

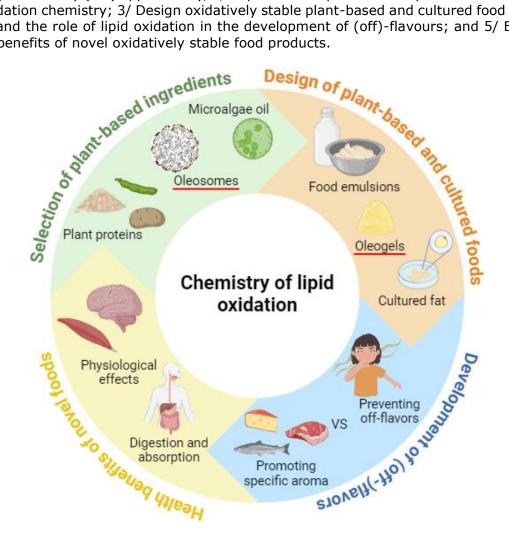
- Production and purification of fungal pectin-active enzymes
- Characterise the biochemical properties of pectin-active enzymes, such as their substrate specificity on extracted pectin and product profile
- Based on literature, review the potential of structurally different pectin-oligomers as prebiotics

Techniques to be used: protein production and purification techniques, HPSEC, HPAEC, MALDI-TOF-MS, UPLC-MS, Alphafold3, protein structure similarity software

Part 5 Chemistry of lipid oxidation in the design of novel foods

Lipids are a diverse group of molecules, including e.g., fatty acids, triacylglycerols, phospholipids, and sterols. They play a crucial role in foods, contributing to the flavour, texture, and nutritional value. One major challenge for food industry to design lipid-based products is lipid oxidation, a process that diminishes the nutritional value of the foods, generates unpleasant off-flavours and compromises the shelf-life of the products. This issue has become even more challenging with the rise of plant-based diets and lab-grown foods, driven by sustainability, health, and ethical concerns. Indeed, little is known about the mechanisms of lipid oxidation in these novel foods or how to control it to develop safe, nutritional and tasty foods.

In the Lipid Team, we tackle this challenge through 5 key aspects: 1/ Develop advanced analytical tools for the characterization and quantification of food lipids and their oxidation products (e.g., Nuclear Magnetic Resonance spectroscopy (NMR), gas and liquid chromatography (GC and LC), and mass spectrometry (MS) approaches), 2/ Explore the impact of novel plant-based ingredients on lipid oxidation chemistry; 3/ Design oxidatively stable plant-based and cultured food products; 4/ Understand the role of lipid oxidation in the development of (off)-flavours; and 5/ Evaluating the health benefits of novel oxidatively stable food products.



Topic 5.1 Are Omega-3 rich microalgae oil a good alternative for fish oil?

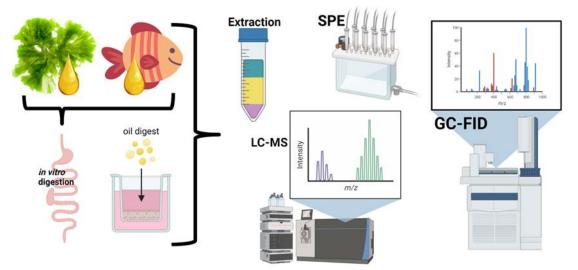


Keywords:Supervisors: Daniëlle WesselsMarie HennebelleHealth, characterization, plant based dietTopic suitable for: BSc & MSc

Do you want to investigate the potential of microalgae oil as a vegan and sustainable alternative for fish oil? Are you interested in learning more about oil chemical characterization and properties? Then, join us in this project!

Long-chain omega-3 polyunsaturated fatty acids (ω 3 LC-PUFAs) are of great interest for their potential health benefits. In foods, they are mostly found in fatty fish, such as salmon, mackerel, or sardine. However, the daily recommended intake of 500 mg/day ω 3 LC-PUFAs is almost never met through the diet. To compensate for the low dietary intakes, people can take fish oil supplements, but the use of fish as source of ω 3 LC-PUFAs for these supplements is questioned due to safety (raising contaminations) and environmental (overfishing) reasons. A proposed alternative is marine microalgae, which appears to be a viable and sustainable source of ω 3 LC-PUFAs, as they do not require freshwater or farmland and have the ability for high fatty acid accumulation. Microalgae oil can also be incorporated in a vegan diet, has less unpleasant odours, and has lower amounts of cholesterol compared to fish oil, but differs in its chemical properties. However, differences in chemical composition in fish and microalgae oils can result in differences in health benefits, partly due to changes in digestion and absorption.

In this project, you will determine the chemical characteristics of ω 3 LC-PUFAs rich oils (either pure or in food matrices) to later correlate their composition to their functionalities and health benefits.



Example of BSc/MSc projects: Determine the chemical composition of ω 3 LC-PUFAs rich microalgae oil and fish oil (pure or in matrix), or oil (matrix) digests using chromatography and mass spectrometry methods.

Techniques to be used: Fatty acid composition by GC-FID, triacylglycerol and oxylipin composition by UPLC-MS, Solid Phase Extraction

Part 6 Flavor chemistry

Flavor is one of the main factors that influences consumers' choice of food. It comprises taste, which is caused by non/semi-volatile tastants, and aroma, which is caused by volatile odorants. Some of these tastants and odorants occur in raw foods and food ingredients, whereas others are formed during processing in industry or during cooking at home. The latter category of flavor compounds is also referred to as 'process flavors' or 'reaction flavors'.

Many of the reactions underlying the formation of process flavors are only partly understood, which makes it difficult to control and steer them. This raises challenges, especially in the development of novel products. Think about <u>plant-based meat alternatives</u>, which are important drivers of the <u>protein transition</u>. When you roast them, flavor is generated, but it does not accurately mimic the flavor of roasted meat, which may prevent meat enthusiasts from switching to plant-based products. Targeted solutions for such flavor challenges can only be developed when having a detailed understanding of the underlying chemistry. Therefore, within the Flavor Chemistry group of FCH, we aim to unravel the complex reactions involved in the formation of process flavors.

In addition to tastants and odorants, <u>flavor-enhancing and flavor-modifying compounds</u> may be formed during food processing and preparation. Such compounds have no inherent flavor, but may alter the perception of taste and smell. Therefore, these molecules can be interesting functional food ingredients, e.g. to enable salt or calorie-reduction without loss of flavor intensity. Within the Flavor Chemistry group of FCH we study the formation and mode of action of such flavor-modifying compounds, and in collaboration with sensory scientists we explore their potential as food ingredients.



Topic 6.1 Taste-enhancing compounds for next-generation meat replacers





Keywords:	Supervisor:	Angelina Hopf	Roelant Hilgers
Protein transition, molecular gastronomy, enzymes, purification		Topic s	uitable for: BSc & MSc

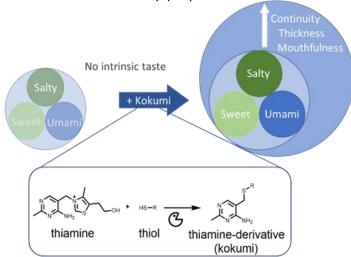
Do you want to find out what kokumi is and how we can use it to make food more flavorful? Are you interested in learning more about enzymatic and chemical synthesis methods? Then, join us and help to explore new enzymatic synthesis methods to produce thiamine-derived kokumi compounds!

Plant-based meat alternatives play an important role in the protein-transition towards a more plant-based diet. Although their quality and market share have increased in the last decades, two major points of criticism are still to overcome which are i) most of them contain excessive amounts of salt, and ii) their flavor does not accurately mimic that of real meat yet.

An interesting approach that might tackle both issues at the same time is the use of so-called kokumi compounds. These compounds are in itself tasteless, but increase the complexity, continuity and mouthfulness of a food. Kokumi compounds have been reported to enhance both saltiness and umami, two important aspects of meat flavor and therefore may boost the perceived meatiness of plant-based meat alternatives, while enabling a reduction in salt content.

The vast majority of kokumi compounds identified so far are small peptides, especially γ -glutamyl peptides, which are typically found in fermented foods. However, thiamine-derived kokumi compounds that show promising low kokumi-thresholds are typically formed through heat-induced reactions, e.g. during simmering of a soup or stew and during roasting of meat.

In this project, we aim to investigate a novel enzymatic route for the formation of thiaminederived kokumi compounds to enable more selective methods to synthesize pure (food-grade) kokumi compounds, to evaluate their sensory properties.



Thesis project:

• Exploration of enzymatic synthesis methods of thiamine-derived kokumi compounds

Techniques to be used: UHPLC-MS, preparative HPLC, Flash chromatography, NMR, molecular biology

Topic 6.2Meat aroma generation in plant-based meat replacers using thermo-
mechanical processing



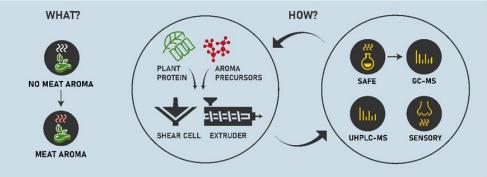


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Keywords:	Supervisor:	Mirjam Hemelaar	Roelant Hilgers
Protein transition, molecular gastronomy		Topics	suitable for: BSc & MSc

Are you interested in how chemical reactions contribute to typical meat aroma? Do you want to understand how these reaction are influenced by thermomechanical processing and plant protein sources? Then help us in our quest to make plant-based meat analogues taste more like authentic meat!

Plant-based meat analogues (PBMAs) play an important role in the protein-transition by offering meat-oriented consumers products that mimic the texture and flavor of meat. The fibrous texture of meat can already be mimicked quite successfully by thermomechanical prosessing of plant-based proteins. Shear cell and extrusion are primary examples of thermomechnical processing. In contrast, it remains challenging to mimic authentic meat flavor. As flavor is one of the key factors in consumers' choice of food, closing this flavor-gap is expected to bring more meat-oriented consumers on board of the protein transition. The unique flavor of meat is obtained through chemical reactions (e.g. Maillard reaction and lipid oxidation) that take place at high temperatures during cooking. As high temperatures (120-160°C) are also applied during the thermomechanical texturization in PBMA production, it may be possible to combine meat flavor formation and texturization in a single process step. We aim to understand which flavor precursors (e.g. sugars, amino acids, vitamins) and reaction conditions (e.g. time, temperature, water content) are required to generate authentic meat aroma during thermomechanical processing of plant proteins.

In this thesis project, we will be focussing on measuring the aroma development using several precursor mixtures during the production of small scale PBMA samples using a small scale way of making texturized protein. In addition, we need to know if the produced aromas are stable when the product is reheated after processing (to stimulate preparation by the consumer at home).



Thesis project:

• Prepare and analyze the aroma profiles of different combinations of precursors in plantbased meat analogues, to explore their potential to mimic authentic meat aroma.

Techniques to be used: Closed Cavity Rheology (CCR), (SPME-)GC-MS, GC-IMS, potentially: Solvent-assisted flavor evaporation (SAFE), shear cell, UHPLC-MS

Topic 6.3 Can heme-containing proteins catalyze meat aroma formation?





Roelant Hilgers

Protein transition, molecular gastronomy, extraction, purification

Keywords:

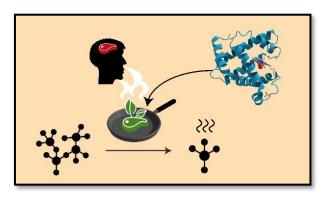
Supervisor: Eva Oehlers

Topic suitable for: BSc & MSc

Do you want to contribute to the flavor of plant-based meat analogues? And do you have a keen interest in understanding chemical reactions? Come help us unravelling the role of heme-proteins in meat aroma generation!

Whereas the texture of plant-based meat analogues (PBMAs) has been improved tremendously over the past years, the flavor is still lagging behind. Many consumers crave the unique flavor of cooked (e.g. roasted) meat. If PBMAs would more accurately mimic this flavor, meat-oriented consumers would find it easier to switch to a plant-based diet, aiding the protein transition. Recently, it has been proposed that heme-proteins, myoglobin and hemoglobin, contribute to meat aroma generation during cooking of meat. Hence, heme-proteins might be useful ingredients to improve the flavor of PBMAs. Although animal-derived heme-proteins are unsuitable as ingredients in vegetarian products, look-a-like proteins can also be found in plants and microalgae e.g. leghemoglobin from soy, and precision fermentation technologies are currently being developed to produce myoglobins in an animal-free way. An added benefit of using heme-proteins in PBMAs is they are red colored in the native state, and turn brown upon heating, contributing to a meat-like appearance.

Although it is possible that heme-proteins somehow contribute to meat aroma generation during cooking, the underlying mechansism(s) are not yet understood. Although they are referred to as 'meat aroma catalysts' in recent studies, evidence for a true catalytic effect in meat aroma formation is lacking. Without a proper understanding of how heme-proteins contribute to meat aroma generation, it remains difficult to optimize their use and application in PBMAs. Hence, this research project aims to sniff out how heme-proteins affect aroma generation in presence of various plant-based (meat) aroma precursors.

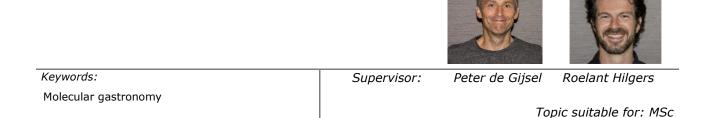


MSc or BSc project:

Extraction and purification of myoglobin, and evaluation of its effect on aroma formation in model systems containing plant-based (meat) aroma precursors.

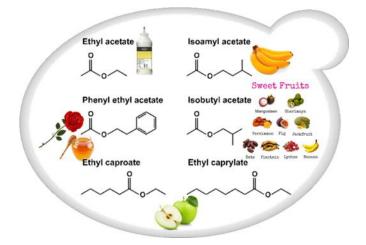
Techniques to be used: (preparative) liquid chromatography, SDS-PAGE, DUMAS, GC-MS, (UHPLC-MS)

Topic 6.4 Decoding the flavor of alcoholic beverages



Do you want to further develop your knowledge in the field of flavor science? Have you always wondered which aroma compounds are responsible for the flavor profiles of wine, beer or distilled beverages? In this project you will help us in cracking the flavor code of such beverages and design an MSc practical course based on your results.

Alcoholic beverages, such as wines, beers and spirits, contains dozens, if not hundreds of aroma compounds. Only a small percentage of these molecules, however, actively contributes to the perceived aroma of the beverage. Most molecules are present at concentrations below their odor threshold or is masked by the presence of other aroma compounds. By combining various sensory and instrumental analyses, using the so-called *sensomics* approach, one can find out which molecules in a complex mixture are responsible for the perceived flavor profile of foods and drinks. In this project you will i) extract aroma compounds from alcoholic beverages, ii) examine their molecular structures and their concentrations in the beverages, iii) develop recipes using pure aroma compounds to mimic the flavor of the beverages, and iv) design datasets and figures based on your results for a Food Technology MSc course.



MSc project:

Decoding the aroma profile of wines and beers using the sensomics approach

Techniques to be used: Solvent-assisted flavor evaporation, GC-FID, GC-MS

Part 7 Design and development of e-learning materials

Our laboratory invests in the design and development of digital learning materials for food chemistry education using a Design Oriented Research Approach (DORA). Several types of learning material were developed.

- Digital exercises / linear cases: intensive questions and tasks with built in feedback and hints to acquire knowledge on food chemistry. This ranges from > 100 exercises for the course Food Chemistry, several linear cases in many courses such as Food Properties and Function and Food Ingredient Functionality. Many of these exercises have been used for several years now and are highly appreciated by our students.
- **Pre-laboratory assignments**: assignments in which students design several experiments to answer a couple of research questions related to food chemistry.
- **Quantitative assignments**: assignments in which students calculate on chemical reactions in food products.
- **Online Problem Based Learning (group work**): by using tools such as Google Docs, Brightspace, or even ExperD we have developed several options to implement online group work based on the principles of Problem Based Learning
- Labbuddy (ExperD and WebLabManual): a design environment for students to design their (own) lab experiments (ExperD), connected to the digital lab manual. Although developed during a PhD project at our group, this program is now hosted by Kryt bv. We still collaborate with them to design and implement new features.
- LabSim (a virtual experiment environment VEE): in this extension of labbuddy students design experiments for certain research questions and then receive the data based on the design choices students made. Students process the data to answer the research questions. VEE can prepare students for lab classes, or even replace (part of) lab classes.

Topic 7.1 Designing (digital) learning activities within the field of food chemistry





An interest in educational theories is	Supervisors:	Bake de Rink	Julia Diederen
required		Topic s	uitable for: BSc & MSc

The (digital) learning environment at the chair group Food Chemistry is continuously in development to improve education. Teachers are always looking for new ways to improve their education, by designing new learning materials or learning activities, and by including new ways of learning. For example, the use of AI or the implementation of academic skills is nowadays an interesting topic to investigate.

The designer of the new learning material is responsible for the content (think of clear instructions, interactive questions, multimedia, digital cases) and guidance (think of hints and feedback) to the students in order to create an effective and motivating learning experience.



As an example, Food Chemistry has invested strongly in pre-lab education to prepare students for laboratory classes. This can be done with a virtual experiment environment (VEE), such as labbuddy, a simulation that covers the complete process of performing research. In our food chemistry education, VEEs enable students to prepare for laboratory education in an effective and efficient way. In some courses VEEs are used stand-alone, without a successive lab class, to let students practice with experimental data within an experimental set-up. Designing an effective VEE is a challenge.

This thesis project can focused around design oriented research: solving a complex educational problem using a design approach. During the educational design, you will gain in debt understanding of the food chemistry topic. You will combine the three fields: food chemistry, educational research, and information and communication technology (ICT). Next to that you need to implement your creativity to create suitable and interesting learning materials. The thesis project can also focus on evaluating an existing learning activity.

Examples of completed BSc thesis topics:

- Designing multimedia for in a virtual experiment environment supporting students in understanding analytical methods
- Design and evaluation of a pre-lab assignment as preparation for a virtual laboratory for the course 'Food Related Allergies and Intolerances'.
- ChatGPT in Food Technology Laboratory education
- Design of feedback and guidance for a digital calculation case
- The effect of prior knowledge and skills on the learning experience of the Laboratory Simulation in the course Food Ingredient Functionality

Thesis projects:

Depending on the interest of the student and the needs of the teachers we can describe the topic for the thesis project together.

- Designing (digital) learning material for activating learning activities for a course in the field of food chemistry.
- Evaluating (digital) learning materials or learning activities within a course in the field of food chemistry.

Topic 7.2 Find the answer to the food chemistry-related question that is already bugging you for long





Supervisors: Bake de Rink

Julia Diederen

Topic suitable for: BSc

In this topic you apply chemistry in the context of agricultural raw materials, food products, or industrial by-products. You are challenged to formulate your own subject. Are you the kind of person that always wonders about the "why" behind chemistry-related phenomena in the context mentioned above? Then, this topic might be very well suited for you! In this thesis project you seek the solution to your own research question.

You should aim at understanding the relation between the composition of a raw material, food, or by-product, the process or storage conditions (e.g. temperature, pH) and the properties (e.g. foaming behaviour) or reactivity (e.g. oxidative stability) of the constituent molecules. Your research question should be in line with this. The experiments that you design should be challenging, yet feasible within the given time span and with the methods available. Be informed that the total amount of experimental time is 2 weeks at the most.



At food chemistry there are methods available to analyse the main components of foods: carbohydrates, lignin, lipids, phytochemicals and proteins. For this BSc thesis topic, you can test your ideas using only the basic laboratory equipment that you already used in the laboratory classes of the courses Food Chemistry, Food Properties and Function or Nutritional Aspects of Foods. During this thesis you will follow the same workshops as are done by the students on other topics.

Possible BSc topic:

 If you are interested in this topic, please contact us to discuss your ideas and feasibility as a thesis before choosing this topic in your top 5: <u>Bake.derink@wur.nl</u> / <u>Julia.Diederen@wur.nl</u>