WAGENINGEN
UNIVERSITY & RESEARCH

L A B O R A T O R Y o f F O O D C H E M I S T R Y

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

Last update: March 2024
Go to www.fch.wur.nl (→ education)

Table of Contents

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 plant-based diet, the development of healthy ingredients and food products, and the valorization of industrial byproducts.

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 regular contact between students and supervisors to maximize the learning curve during your thesis.

Bachelor thesis

Bachelor thesis projects seek a balance between laboratory work and desktop study (data analysis, literature), depending on the topic and your own preferences. You can design and conduct your own experiments within the framework of your supervisor's research, or perform data analysis (using literature data or laboratory results gathered by your thesis supervisor) followed by (a modest amount of) laboratory work for which you design your own experiments based on the outcome of the data analysis.

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.

Workshops

You will have multiple workshops to boost your skills in e.g. data management, literature search, academic writing and presenting, and research design.

Master thesis

Master thesis topics comprise laboratory work in which you design your own experiments and generate data yourself, followed by interpretation of these data and condensing them into a scientific report. Thesis rings will be held once every two weeks, to improve your writing skills. You get the opportunity to work with advanced analytical equipment.

Thesis topics at Food Chemistry

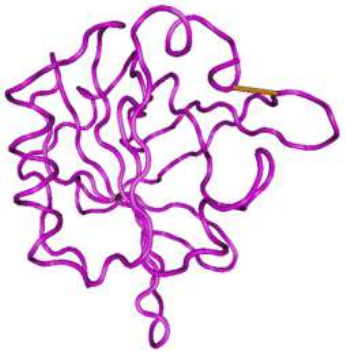
The main research topics of our Laboratory are (see also Table of Contents):

- Part 1: Food proteins
- Part 2: Plant bioactives
- Part 3: Phytochemicals
- Part 4: Food carbohydrates
- Part 5: Lignocellulosics
- Part 6: Food lipids
- Part 7: Flavor chemistry
- Part 8: Educational development

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 this 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 biochemical and physicochemical 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. Additional factors of influence for the functionality of proteins are isolation procedure, (physico-) chemical or enzymatic modification, and the composition and processing route of the food in which they are applied. Knowledge of structure-function relationships of proteins in foods, and the interaction between proteins and other food constituents form the basis for the development of modern processes, of new ingredients and of higher quality products.

In the group there are four major research lines:

1) **Novel and current plant proteins (including microalgae)**: Focussed at isolation methods and issues and characterization of the obtained concentrates/isolates.

2) **Enzymatic hydrolysis of proteins**: To describe the protein hydrolysis a method has been developed focussing on the identification and quantification of all peptides in hydrolysates taken during hydrolysis. This allowed further development of concepts to characterize this process and relevant molecular properties of both protease (selectivity) and substrate.

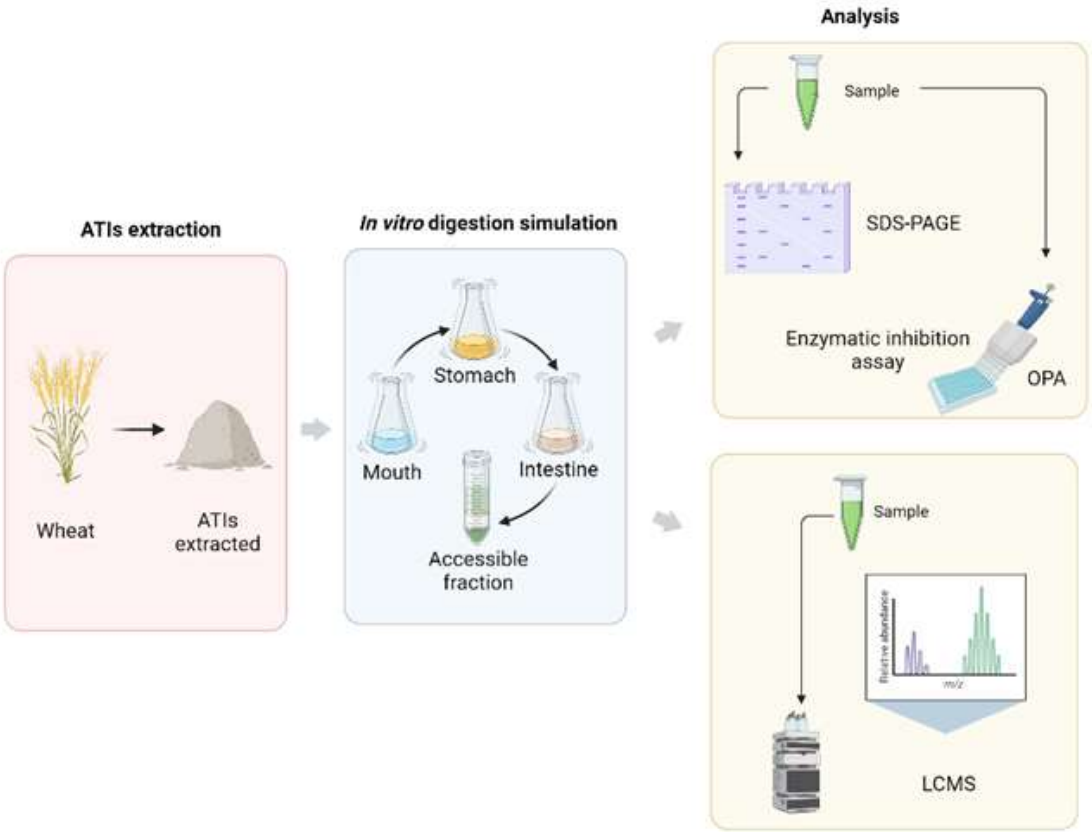
3) **Maillard reactions**: In order to understand the effects of Maillard reactions on ingredient functionality we need adequate methods to quantify the extent of modification. For this different methods are applied, improved and combined.

4) **Foam- and emulsifying properties of proteins**: We develop methods and concepts to describe the these properties in terms of their molecular properties.
Analytical methods used therefore range from those to determine protein conformation (CD), LC-MS, light-scattering and multiple chromatographic or electrophoretic techniques. Asymmetric flow-field flow fractionation coupled with a multi-angle light scattering detector is used for the characterisation of protein aggregates. Furthermore we have equipment to determine interfacial, foaming and emulsifying properties of proteins. This is supplemented with studies of thin liquid films studied under reflected light microscopy. To study *in vitro* hydrolysis we have several pH stat units. The study of proteins from several angles provides a complete overview.

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

Specializations: *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *E: Food Digestion and Health* *F: Gastronomy Science*	*Supervisors:* *Dounia Krouch* *Gijs Vreeke* *Topic 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 α-amylase, key digestive enzymes, ATIs actively contribute to the plant's innate defense against pests and pathogens. These proteins have recently been affiliated with the irritable bowel syndrome (IBS) and non-celiac wheat sensitivity (NCWS). 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 digestion. To do so, first, the ATIs need to be extracted and simulated *in vitro* digestions will be performed. Later, techniques, such as mass spectrometry, the OPA assay, and SDS-PAGE will be used to analyze the digesta. The results will help enhance understanding of ATIs' involvement in NCWS and IBD.



MSc thesis project:

- Understand the stability of ATIs throughout digestion.

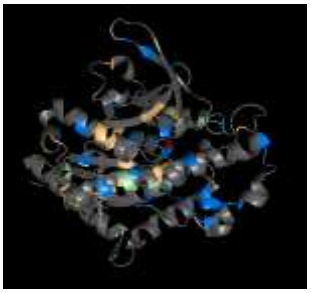
Topic 1.2 Inhibiting enzymatic activities in plant protein isolates


Specializations:	*Supervisors: Thore Diefenbach Gijs Vreeke*
A: Product Design and Ingredient Functionality *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Topic suitable for: 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 techno-functional properties.





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 their functional properties.

BSc. project:

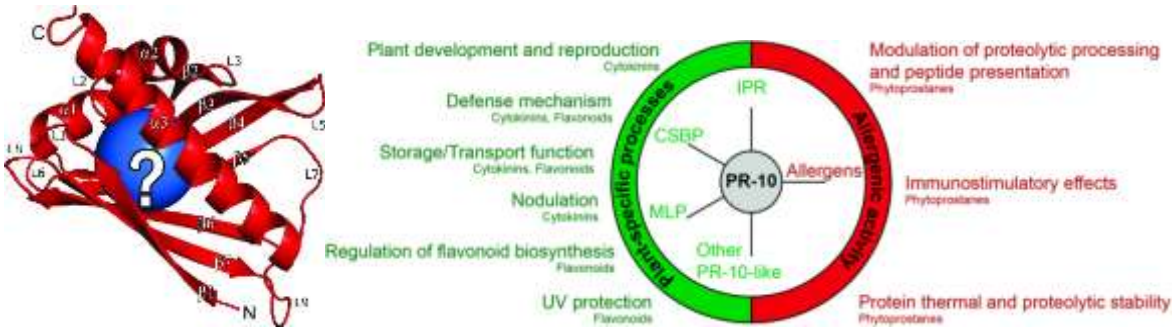
- Investigate by analysis of available data, the approaches used for other plant protein sources (soy, sunflower, lentil) to reduce the enzymatic activities of endogenous enzymes and propose ways to apply these approaches to potato proteins.

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

Topic 1.3 Molecular characterisation of the cashew nut PR10 proteins


Specializations: *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Supervisors:* *Shanna Bastiaan-Net* *Jean-Paul Vincken* *Topic suitable for: MSc* *Collaboration with WFBR and Avans Hogeschool 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).

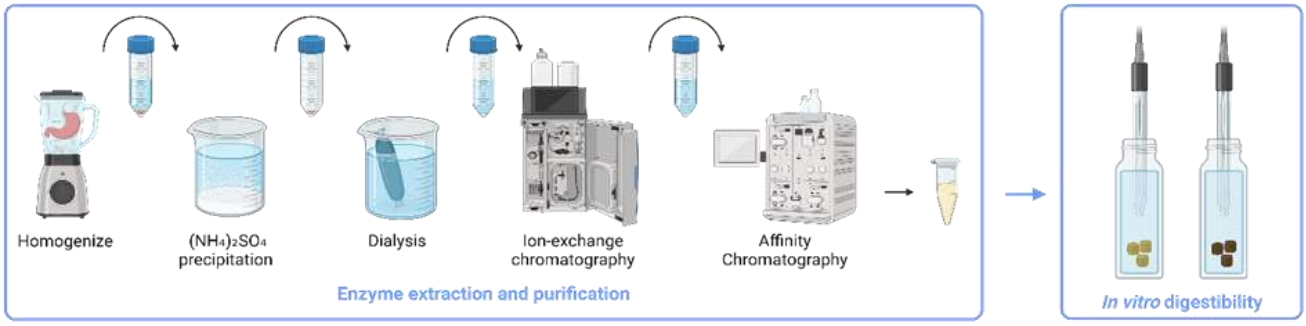
Techniques to be used: SDS PAGE, protein isolation, ANS assay, in vitro digestion

Topic 1.4 Extraction, purification and characterisation of fish proteases to understand the effect of heating on protein digestibility

Specialization: *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Supervisors: Abel Roijakkers Gijs Vreeke* *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 anti-nutritional factors and thereby positively affect protein digestibility. However, heat treatments can also induce chemical reactions such as Maillard reactions (non-enzymatic browning) and protein cross-linking, which have been linked to a 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 Maillard reaction and protein cross-linking on protein digestibility in fish.

First results from *in vivo* digestibility trials suggest, in contrast to in mammals, limited or no effect of Maillard reaction and protein crosslinking on protein digestibility in fish. This might be attributed to differences in enzymatic protein digestion between fish and mammals. The role of proteases *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 operate 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:

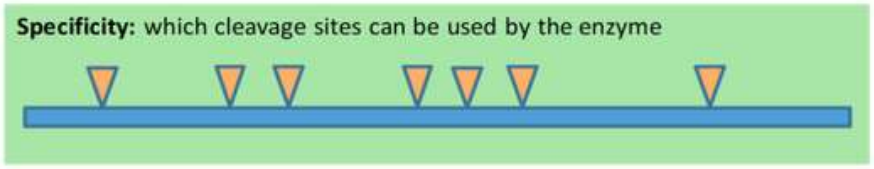
- Extraction, purification and characterisation of fish proteases for *in vitro* assessment of the effect of Maillard reaction and protein crosslinking on enzymatic protein digestion in fish.

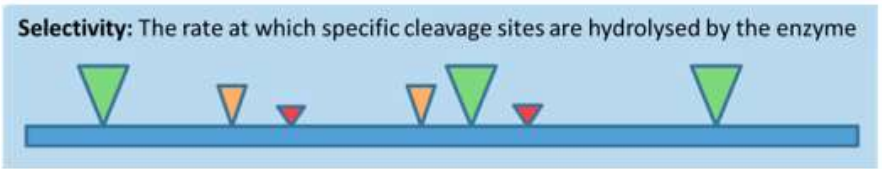
Topic 1.5 Predicting hydrolysis by digestive enzymes using *in silico* docking

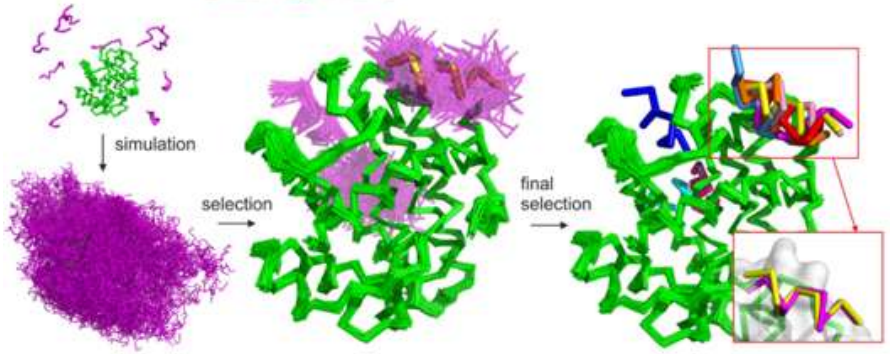
Specialization: *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *D: Dairy Science and Technology* *E: Food Digestion and Health* *F: Gastronomy Science*	*Supervisors:* *Gijs Vreeke* *Jean-Paul Vincken* *Topic suitable for: MSc*

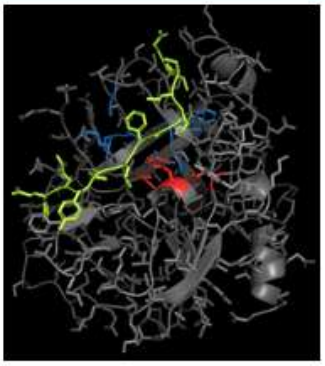
Understanding the enzymatic hydrolysis of proteins is important in the development of products as infant formula, sports nutrition and animal feed, as well as to understand how food is digested.

Last 10 years, we developed a method in the laboratory to determine the individual hydrolysis rates of cleavage sites in a protein. We observed that digestive endo-proteases as trypsin and chymotrypsin do not hydrolyse all bonds efficiently within their specificity. Some of these "missed-cleavages" can be explained using the amino acids around the cleavage site. For instance, bovine trypsin is hindered when charged residues are next to the cleavage site. However, in a lot of cases, we cannot explain the hydrolysis rates using the sequence of the substrate. In this thesis topic, you will explore whether we can use 3D-docking of the substrate to the active site of proteases as tool for predicting hydrolysis. First, various docking tools need to be compared and validated. Later, existing data of peptide release kinetics will be used to unravel the activity of proteases as trypsin, chymotrypsin, pepsin or rennin. New insights can later be validated by hydrolysing new substrates in the pH-stat and analysing peptide release kinetics with LC-MS.



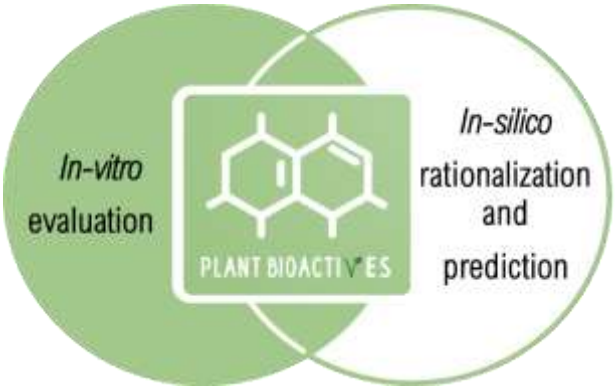






Techniques to be used: ***in silico docking*** *(80-90 %) pH-stat, LC-MS.*

Part 2 Plant Bioactives



Plants produce an enormous variety of secondary metabolites (phytochemicals). This rich structural diversity confers phytochemicals interesting biological activities. Currently there is an increasing demand for natural, healthy and sustainable food ingredients. On top of that, antimicrobial persistence 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. Many of these phytochemical (e.g. phenolic compounds, glucosinolates, saponins) 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 microorganisms is to design combinations of multi-targeted antimicrobials, just like plants do when exposed to pathogens. To create effective synergistic combinations, 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 pump 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) perform a predictive assessment 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 scaffolds; (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 and MS-based (un)targeted analysis (in collaboration with other groups). *In-silico* tools include prediction of molecular properties, 3D pharmacophore modelling and molecular docking.

Topic 2.1 Novel Plant(like)-derived Antimicrobial Modulators

Specializations: *BFT* *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Supervisors: Adrian Kopf Carla Araya-Cloutier* *Topic suitable for: BSc & MSc*

Multidrug-resistant bacteria are a growing and severe threat to society. The indiscriminate use of antibiotics, especially in the animal husbandry sector (e.g. poultry), has exacerbated the problem. Simultaneously, few truly new antimicrobials are being discovered. Therefore, there is a dire need for novel antimicrobials and/or antimicrobial modulators.

Marine environments, and specifically, seaweeds (macroalgae) are excellent sources to look for such compounds. Due to the unique and harsh environment seaweeds grow in, they produce various bioactive chemicals with novel chemical scaffolds and elemental compositions. Seaweeds are already being used in the animal husbandry sector, i.e., as chicken feed, as a natural and eco-friendly antimicrobial agent. However, there is a knowledge gap on active substances, how they interact with bacteria to inhibit their growth and the interplay with antimicrobial-resistant microorganisms.

These projects (see Figure 1) will help discover and understand innovative antimicrobial modulators that can be applied to chicken feed in order to contribute to a more sustainable food system, reduce the disease burden of food-borne pathogens and fight AMR in a One Health approach.

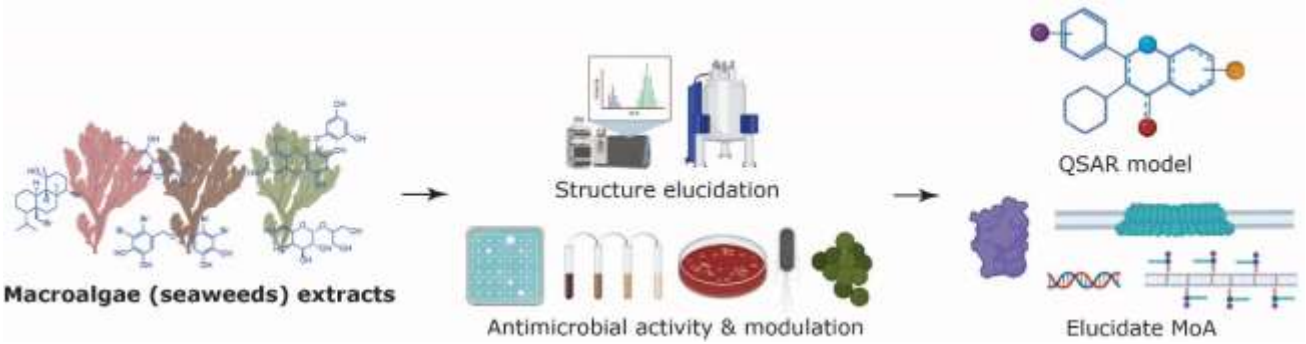


Figure: Schematic overview of projects to discover and understand macroalgae and their derived extracts/bioactive constituents as novel antimicrobial modulators

Thesis projects:

- All projects involve the chemical and microbiological characterization of bioactive antimicrobial modulators from seaweeds. Furthermore, the project will focus on one of the following topics:
  - Establishment of their quantitative structure-activity relationships (QSARs).
  - Elucidation of the mode of action (MoA) with respect to the modulation of pathogenic bacteria.
  - Determination of the bioactivity of the compounds on antimicrobial resistant (AMR) bacteria.

Techniques to be used: (Analytical Chemistry and Microbiology), LC-MS, flash-/prep-HPLC, NMR, antimicrobial activity assays (NAT, MIC, MBC), and in-silico modelling

Topic 2.2 Prenylated (iso)flavonoids: characterizing mode of antimicrobial action using liposomes as model system


Specializations: *BFT* *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Supervisors: Janniek Ritsema Carla Araya-Cloutier* *Topic suitable for: BSc & MSc*

To combat antimicrobial resistance, novel and effective alternatives to traditional antimicrobial agents need to be developed. Prenylated (iso)flavonoids are potent antimicrobial compounds produced by plants of the Leguminosae family. Prenylation refers to the substitution with a hydrophobic five-carbon isoprenoid unit (shown in red in **Figure 1**), a structural feature typically associated with increased antimicrobial activity. The position and configuration of the prenyl group(s) influences antimicrobial activity, in addition to the (iso)flavonoid backbone and presence of other decorations (*e.g.* hydroxyl groups).

To effectively apply prenylated (iso)flavonoids as novel alternatives to traditional antimicrobial agents, deeper insights into their mechanism of action are required. The microbial cytoplasmic membrane is considered one of the first targets of prenylated (iso)flavonoids. Previous research has shown that several prenylated compounds are able to disrupt membrane integrity by permeabilization. However, not all active prenylated (iso)flavonoids permeabilized the cytoplasmic membrane, suggesting other membrane-related effects (*e.g.* rigidification or depolarization) could also play a role in the mechanism of action of prenylated (iso)flavonoids.

This project aims to investigate the membrane permeabilization properties of prenylated (iso)flavonoids using model membranes. Liposomes will be used to simulate the bacterial membrane and understand how membrane composition may affect the activity of prenylated (iso)flavonoids (see **Figure 1**). Furthermore, structure-activity relationships (SARs) may be obtained by testing different prenylated (iso)flavonoids.

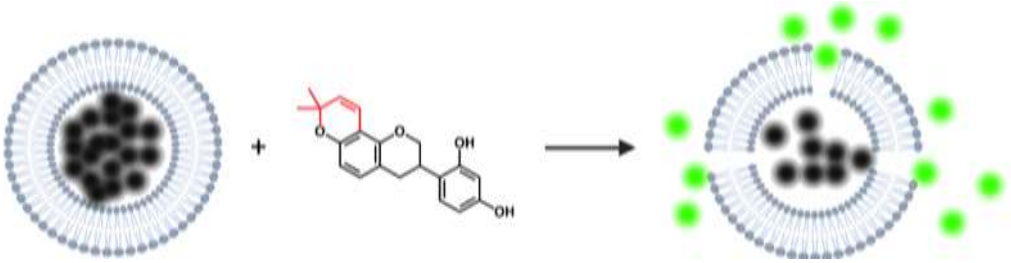


Figure 1. *Liposomes as a tool to study the ability of prenylated (iso)flavonoids to permeabilize bacterial membranes. The liposomes are loaded with carboxyfluorescein, which is fluorescent when released from the liposome.*

Thesis project:

- To gain further insights into the mechanism of antimicrobial action of prenylated (iso)flavonoids by studying the permeabilization of carboxyfluorescein loaded liposomes.

Topic 2.3 Using plant-defence strategies to enhanced antimicrobial properties of phenolics



Specializations:	Supervisors:	*Frenly Wehantouw*	Carla Araya-Cloutier	*Wouter de Bruijn*
BFT *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*			*Topic suitable for: BSc & MSc*	

Novel natural antimicrobials are in high demand due to the increasing antimicrobial resistance (AMR) of microorganisms in healthcare but also in our food and farm production systems and environments. 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, valorization of bioactive compounds from plant by-products (e.g. roots, stem, leaves) is of high interest to increase sustainability of our current food production systems.

Plants contain secondary metabolites that have a wide variety of functions. One of those functions is as defense compounds. These defense compounds are produced in the plant in response to stress and can possess potent antimicrobial activity. Two promising the classes of secondary metabolites are stilbenoids and (iso)flavonoids, which are produced, amongst others, by plants from the Fabacea family. 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 licorice (*Glycyrrhiza* sp.), osage orange (*Maclura pomifera*), and peanut (*Arachis hypogaea*).

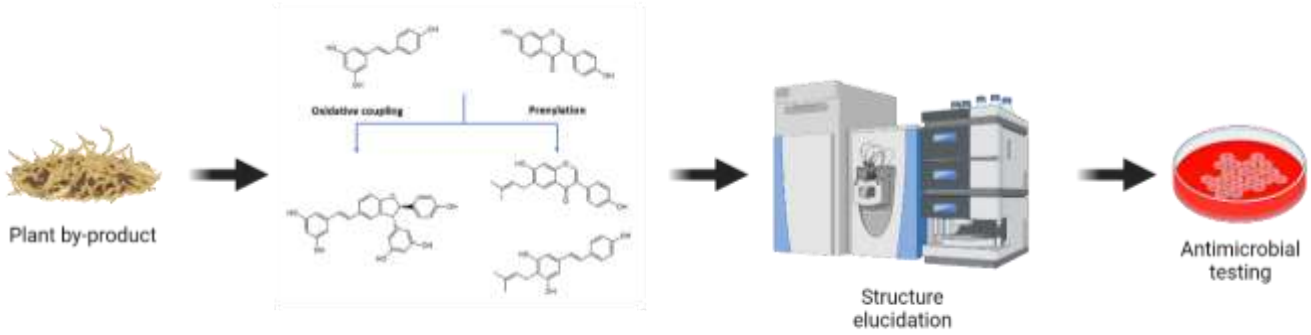


Figure 1. Schematic overview of the project

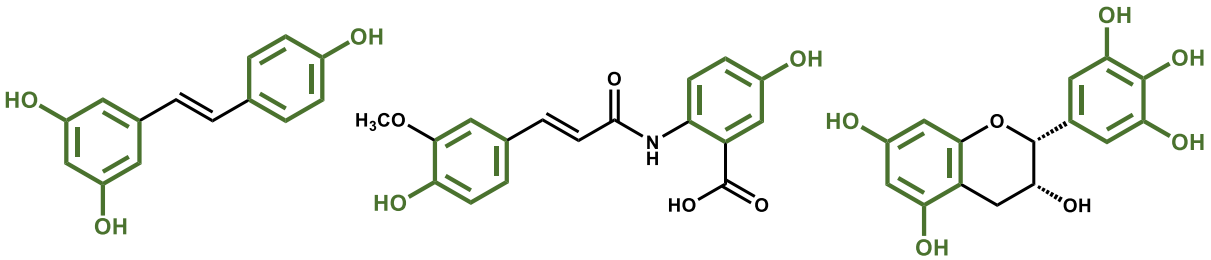
Possible projects:

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

- Investigate antimicrobial activity of dimeric prenylated phenolics from plant by-products (BSc thesis).
- Investigate the enzymatic oxidative coupling of naturally present prenylated phenolics from plant by-products, and the antimicrobial activity of the coupling products (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 (e.g. flavan-3-ols and isoflavones), stilbenoids (e.g. resveratrol), lignans and lignanamides (e.g. hordatines), (hydroxy)cinnamic acids (e.g. coumaric acid, ferulic acid), phenol-amides (e.g. avenanthramides), triterpenoid glycosides (e.g. saponins), and carotenoids. 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.



Resveratrol (stilbenoid) **Avenanthramide 2f** (phenolamide) **Epigallocatechin** (flavan-3-ol)

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. Thus, 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 Bioactives Group.

Topic 3.1 Plant saponin-protein interactions: a challenge in the protein transition

Specializations: *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science*	*Supervisors:*	*Pauline Damhof*	*Wouter de Bruijn* *Topic suitable for: MSc*

The rising interest towards protein sources derived from plant material compared to the traditional animal-sourced protein is commonly known as the protein transition. Currently, one of the main challenges within the protein transition is the presence of undesired molecules, which have off-flavours and/or anti-nutritional properties, in plant protein products.

Saponins are generally recognized as the most important culprits for off-flavour, since they are responsible for a bitter, astringent, and metallic off-taste. They are naturally present in the majority of plant materials. Saponins consist of a triterpenoid or steroidal aglycone with one or more sugar units attached (figure 1).

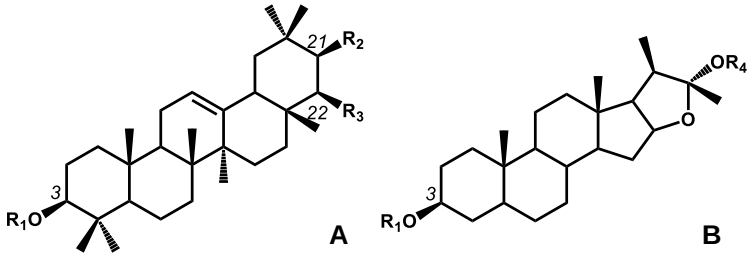


Figure 2 Schematic structure of A) triterpenoid saponin and B) steroidal saponin

Due to saponin-protein interactions, saponins are co-extracted during isolation of proteins, which is why they end up in plant protein concentrates and even in isolates. The presence of saponins and their interactions with proteins can affect the properties of the plant proteins when applied in food products. Besides off-flavour, this can results in reduced protein digestibility, which is why saponins are commonly considered to be antinutritional factors.

Literature suggest that the type of backbone, as well as the diversity in sugar moieties plays an important role in formation of saponin-protein interactions. However, to date, a systematic evaluation of this structure-interaction relationships has not been conducted. The aim of this project is to gain more insight in the plant saponin-protein interaction, as well as the effect of saponin structure on the interactions. If we better understand saponin-protein interactions, then we can improve flavour, purity and quality of plant protein concentrates and isolates, thereby contributing to the protein transition.

MSc thesis projects will involve:

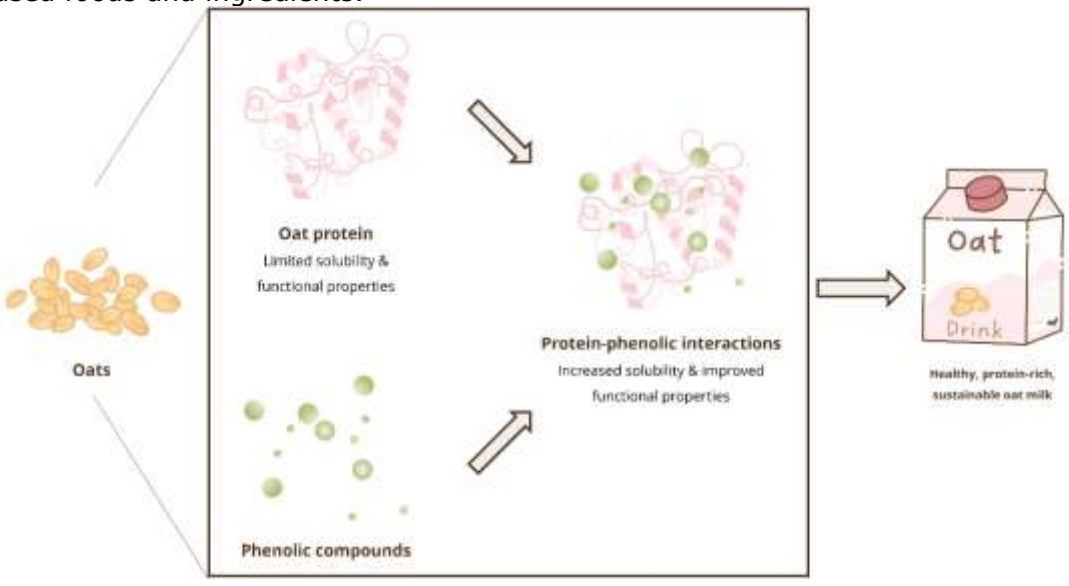
- Characterisation of (modified) saponins with advanced analytical techniques (e.g. UHPLC-PDA-MS)
- Investigation of the interaction between saponins and plant-protein materials.

Topic 3.2 Increasing protein solubility by understanding protein-phenol interactions

Specializations:	*Supervisors:* *Solange Ha* *Wouter de Bruijn*
A: Product Design and Ingredient Functionality *C: Food Fermentation and Biotechnology* *F: Gastronomy Science*	*Topic suitable for: MSc*

There has been an increased consumer demand for healthy, sustainable foods with high-protein content. This has stimulated the food industry to find alternative plant-based protein sources. An alternative protein source could be oats, due to their relatively high protein content of 15-20%. One of the main challenges in application of oat proteins is their limited water solubility, resulting in limited functional properties at the food-relevant pH range of 4-7. This is also reflected in commercially available oat milks, which 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, forming protein-phenolic complexes and conjugates, respectively. Various parameters have been proposed to affect these interactions, in particular the pH. However, most of the information on protein-phenolic interactions is based on studies with phenolics and soluble dairy proteins. A potential downside of these interactions is that they could reduce protein digestibility.

The aim of this project is to increase the protein solubility of oat by modifying the protein with phenolic compounds from oat, without negatively affecting digestibility. 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 plant-based foods and ingredients.



Possible MSc projects:

- Modification of oat protein with various phenolic compounds, and characterization of the modified proteins and phenolic compounds using advanced analytical methods (e.g., LC-MS).

Topic 3.3 The role of plant-based protein ingredients in stabilization of emulsions against oxidation



Supervisors: *Quirine Hafkamp* *Wouter de Bruijn & Marie Hennebelle*

Specializations:	*Topic suitable for: BSc & MSc*
BFT *A: Product Design and Ingredient* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	

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 plant-derived 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, 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.

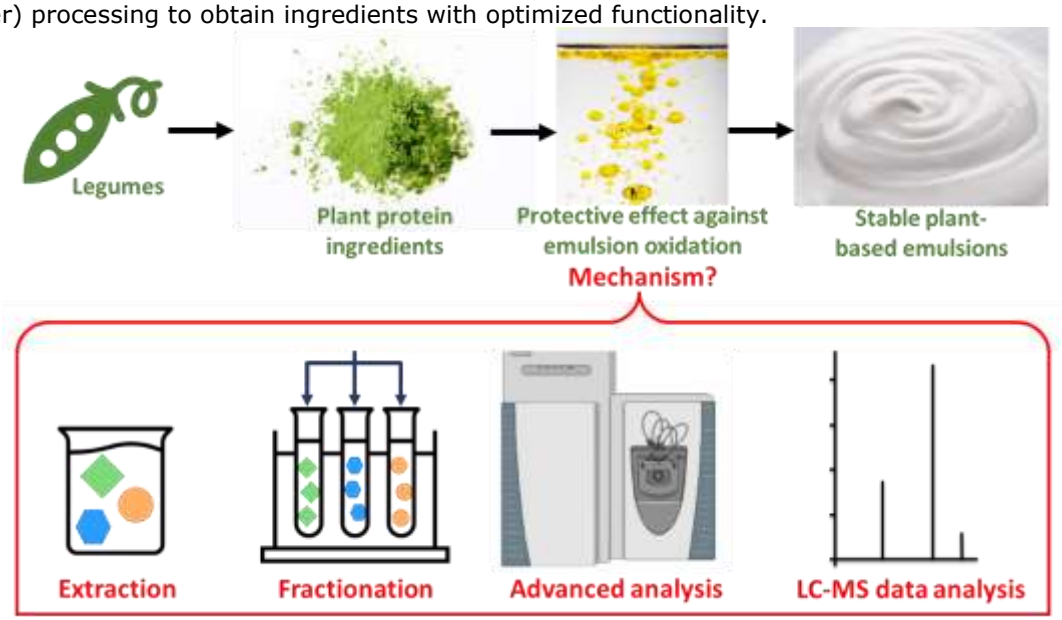


Figure 3: Overview of this study. The main activities for thesis projects are show in red.

This is a new project, thus details of possible BSc and MSc thesis projects are still to be determined.

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.

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.

Part 4 Food carbohydrates

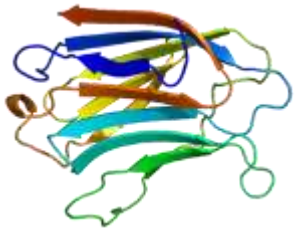
Carbohydrates determine to a large extent quality attributes of the final food product while polysaccharides (e.g. pectic substances, hemicelluloses, cellulose) as present in fresh fruits and vegetables (e.g. ripeness, texture) determines their typical characteristics as well as their processing characteristics in the manufacture of foods (juices, nectars, purees, preserves). Polysaccharides also influence the extractability of important constituents of plant raw materials like sugar, oil, proteins, etc. Dietary fibers and prebiotic oligosaccharides, including mammalian milk oligosaccharides, play an important role in human and animal health, and their behaviour in the gastrointestinal tract strongly depend on the chemical structure of these fibers.

In general, various classes of oligo- and polysaccharides as present in fruits, vegetables, cereals or food products derived here from and of agrotechnological by-products are extracted and characterised by e.g. sugar (linkage) composition, substituents, molecular weight. Unknown carbohydrate structures are separated by (preparative) chromatography and characterised using mass spectrometry and NMR. Enzymatic fingerprinting methods using pure and well characterised enzymes are being used and further developed to enable 'sequencing' of complex carbohydrate structures using state-of-the-art LC-MS platforms. The fate of individual prebiotic and dietary fiber structure during the digestion and fermentation in *in vitro* models as well in human and animals are monitored using the same analytical techniques.
Relationships between the chemical fine structure of the carbohydrate under investigation and the corresponding functional property of this carbohydrate (isolated or as present in the original product) will be established**.**
Typical materials studied are: cereals like wheat, corm; fruits and vegetables like apple, tomato, carrots, potatoes, soybeans; food ingredient like pectin, galactomannans, xanthan, as well as human milk and fermentation digests.

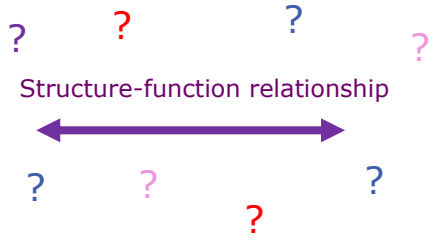
Topic 4.1 Unravelling the structure-activity relationship of rhamnogalacturonan-I rich pectins and Galectin-3

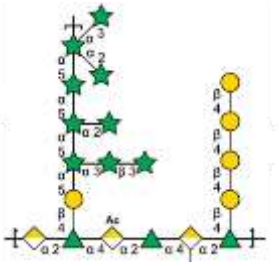

Specializations: *BFT* *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *E: Food Digestion and Health* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Supervisors:* *Anne Kleijn* *Carolina Pandeirada* *Topic suitable for: BSc & MSc*

Galectin-3 is a carbohydrate-binding protein that is upregulated in individuals with cardiovascular disease, inflammatory diseases, and a number of cancer types. Therefore, this protein is a promising target for the prevention and/or treatment of these diseases. Galectin-3 is synthesized and found throughout many types of cells, can be transported extracellularly, and exerts its many functions through binding to glycan ligands.
Modified pectins, rich in complex rhamnogalacturonan-I (RG-I) and of diverse origins, have been shown to bind to galectin-3 and prevent fibrosis. The structure-function relationship between these active pectins and galectin-3 remains unclear. In addition, as pectin is generally considered a non-digestible fiber, whether the polysaccharides are intact when they bind, as well as the location of binding in the body, is unknown. In order to unravel the binding activity behavior of pectins on galectin-3, it is vital to determine to what extent pectin or its fragments can be recovered in the relevant locations in the body.



Galectin-3





RG-I-rich pectin

This project aims to elucidate the structure-function relationship between pectin and galectin-3. This includes extensive structural analysis of the pectin as well as method development to study the fate of pectin in the human body.

Possible BSc project:

- *Investigate the binding of pectins to galectin-3*

Possible MSc projects:
In this project, you will study the complex molecular structure of RG-I-rich pectins using a variety of techniques (e.g. HPSEC, HPAEC, GC, HILIC-MS)

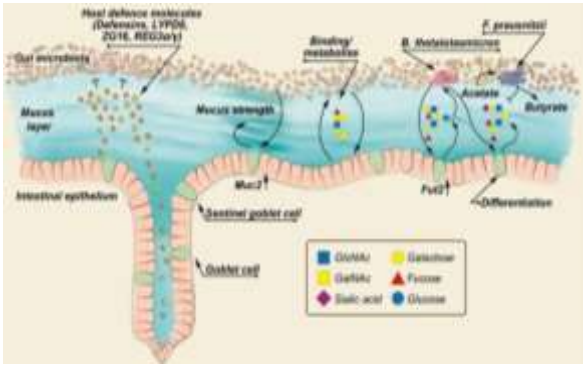
- Chemical characterization through structural analysis of galectin-3 binding pectins obtained from pumpkin
- Modification of pectin through enzymatic or chemical treatment and subsequent structural analysis

Topic 4.2 Revealing mucus sugar composition and its relation with microbiota and diet

Specialisations: *BFT* *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *E: Food Digestion and Health* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	Supervisors:	*Carol de Ram*	*Carolina Pandeirada*

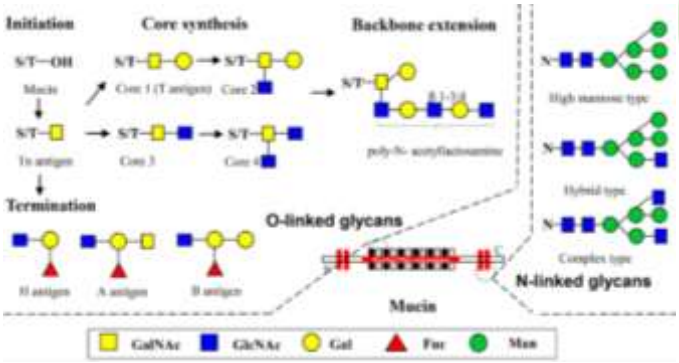
Topic suitable for: BSc & MSc

The intestinal mucus (slime) layer is essential for a healthy gut. This layer contains O-linked and N-linked sugars or glycans that have an essential role in interacting with the rich and diverse gut microbiota. Moreover, the mucus layer is also the first line of defence against bacteria and viruses (disease). To note, a properly "functional" mucus layer can be altered via the diet (e.g. fibre intake) (Arike et al. 2017). The interactions between the mucus layer, the microbiota, and the diet are highly complex and still poorly understood (Qu et al. 2019).



Schematic overview of the mucus layer demonstrating the interactions between the microbiota (Schroeder et al. 2019)

In this study the aim is to 1) unravel the sugar composition of the gut mucus and 2) understand the interaction between mucin glycans microbiota, nutrients, and diseases. We do this by 1) characterisation and quantification of glycans in *in vitro* grown mucus (Elzinga et al. 2021) and 2) studying the (enzymatic) degradation of mucins by gut associated bacteria.



Overview of the structure of mucins highlighting the O-glycans and N-glycans (Qu et al. 2021)

Possible MSc projects:

- Method optimisation for the release and (isomeric) analysis (UPLC-MS/MS, MALDI-TOF-MS, IMS) of O-linked and N-linked sugars from mucus/mucin samples. This could be commercial samples or *in vitro* samples (exposed to dietary fibre/cytokines).
- Gain more insight in glycan binding sites, linkage preference, as well as expressed enzymes in order to unravel mechanisms of glycosylation in mucins and between the gut microbiota and the mucin glycans (HPLC, UPLC-MS/MS, MALDI-TOF-MS, IMS). This could be commercial samples or *in vitro* samples (exposed to dietary fibre/cytokines).

Topic 4.3 Multidimensional mass spectrometric characterization of oligosaccharides

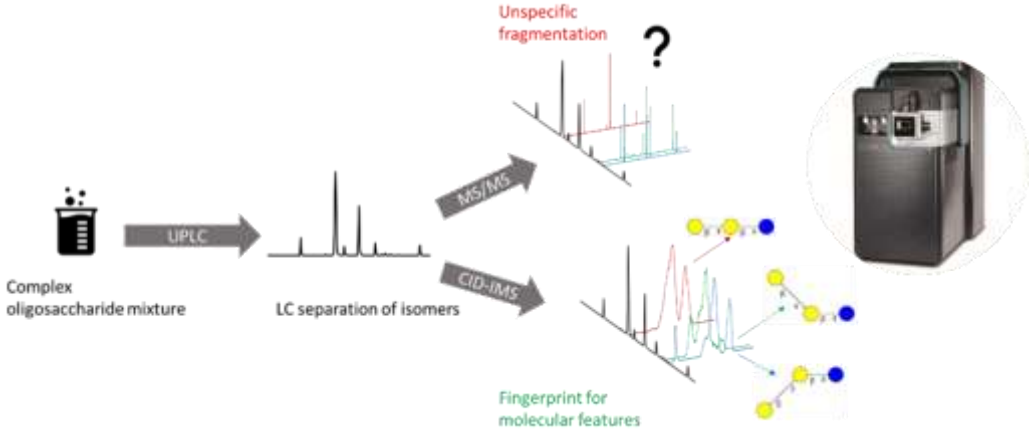


Specializations:	*Bram van de Put*	*Carolina Pandeirada*	*Wouter de Bruijn*
BFT *A: Product Design and Ingredient Functionality* *B: Dairy Science and Technology* *C: Food Digestion and Health* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Topic suitable for: BSc & MSc*		

The detailed molecular structure of oligosaccharides dictates their properties, but no analytical technique can unravel them in a reasonable time. If we can solve this problem, the impact for research fields like nutrition, medicine, and even biobased materials would be immense. Using a combination of chromatography, ion mobility spectrometry and mass spectrometry (LC-IMS-MS) we try to solve the intriguing puzzle of structural variation in oligosaccharides. Are you interested in the structural characterization of complex mixtures? Do you want to use the most advanced analytical equipment? And (most importantly) did you get interested by what you've read thus far? Then this might be the project for you!

The complete sequence of oligosaccharides as present in complex mixtures featuring differences in size, monosaccharide type, linkage type, and anomeric configuration. Currently, these structure can only be characterized via NMR spectroscopy after extensive chromatographic purification. This process can easily take days (!) for each individual compound. For oligosaccharide mixtures consisting of over 50 unique compounds, full structural characterization by NMR is thus out of the question.

In contrast, LC-MS is able to separate and detect all compounds within a mixture in a matter of hours, but does not (yet) provide sufficient structural information for isomers. Ion mobility spectrometry adds (gas-phase) isomeric separation of (fragment-) ions within the MS to LC-MS approaches but much remains to be done to effectively apply this method.



BSc projects:

- Investigation of current LC, IMS, and MS strategies for oligosaccharide characterization

Possible MSc projects:

- Recording a calibrated database of GOS disaccharide IMS spectra
- studying the fragmentation of trisaccharides to disaccharides

Topic 4.4 Levelling Up Tempeh: Unravelling the Structure of Antidiarrheal Extracellular Polysaccharides

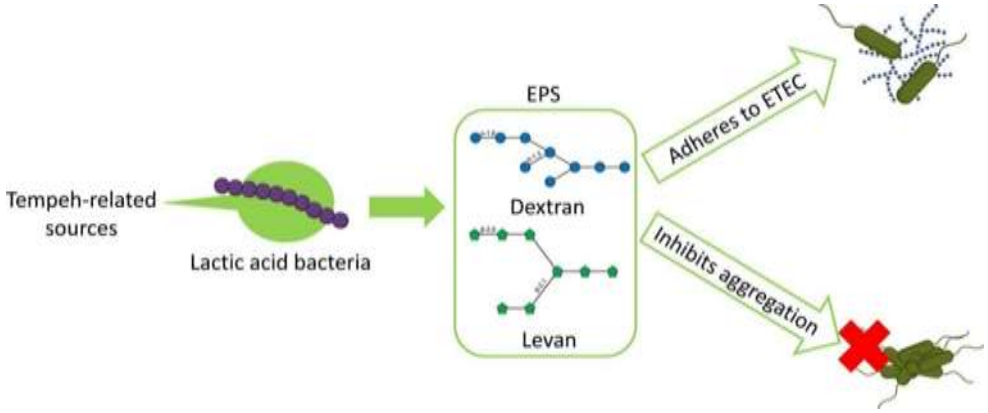


Specialization:	*Supervisors:* *Theodorus Eko Pramudito* *Cynthia Klostermann* *Carolina Pandeirada*
A: Product Design and Ingredient Functionality *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Topic suitable for: MSc*

Background: Enterotoxigenic *Escherichia coli* (ETEC) can cause diarrheal disease in humans and other mammals through adhesion on the intestinal epithelial surface which is followed by toxin production. Inhibition of ETEC adhesion to epithelial surface can thus reduce the incidence of diarrhoea. It has been reported that some bacterial extracellular polysaccharides (EPS) display anti-adhesion bioactivity against ETEC. Tempeh is a soy-based fermented food product that contains lactic acid bacteria (LAB). We have isolated LAB strains from tempeh with EPS-producing capability that can inhibit ETEC adhesion to mammalian intestinal mucin. Currently, we are characterizing the chemical structural feature of the EPS that play an important role in anti-adhesion bioactivity and whether EPSs can be produced when the LAB isolates are grown on soy-based substrate.

Approaches: In this project, you will investigate the structural characteristics of either EPSs produced during the fermentation of soy-based substrate (tempeh) or water-insoluble EPS produced by tempeh-associated LAB on defined substrate. You will investigate the sugar composition of EPSs, their molecular weight, and glycosidic linkages. The EPS can also be subjected to chemical and enzymatic modifications to see the effect of a certain structural feature to anti-adhesion bioactivity. After we have revealed the chemical structure of the EPSs, they might be subjected to various bioassays to study their potential as anti-diarrheal agent.

Relevance: This project is expected to result in the development of tempeh as functional food for diarrhea prevention. Elucidation of bioactive EPS structure can open the opportunity for alternative antidiarrheal medication other than the use of antibiotics.



MSc project (student can choose one of the following):

- Characterization of EPS produced from soy and tempeh fermentation.
- Structural elucidation of water insoluble EPS produced by tempeh-associated LAB

Techniques to be used: HPAEC, HPSEC, MALDI-TOF/MS, NMR, enzymatic modification of carbohydrates, mucin adhesion assay.

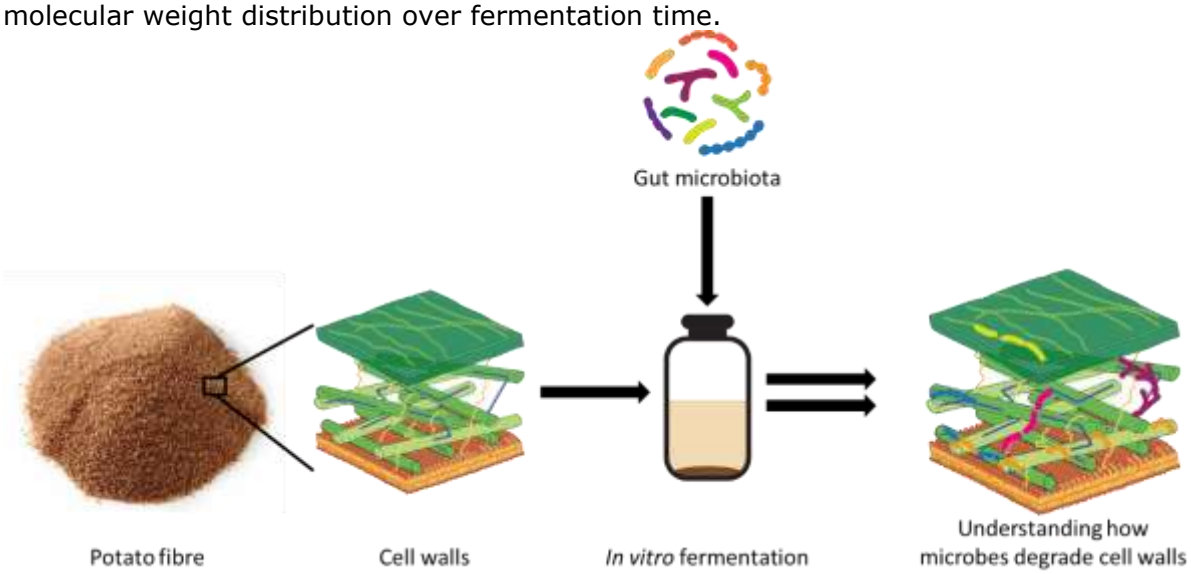
Knowledge in microbiology laboratory techniques is mandatory!

Topic 4.5 Understanding the microbial degradation of complex dietary fibres in the gut


Specialization:	*Supervisors:*
A: Product Design and Ingredient Functionality *C: Food Fermentation and Biotechnology* *E: Food Digestion and Health* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Cynthia Klostermann* *Carolina Pandeirada* *Topic suitable for: MSc*

Dietary fibres are compounds resistant to digestion in the upper gastro-intestinal tract to be fermented in the colon by gut microbiota. Gut microbiota possess many different enzymes able to degrade a wide range of dietary fibres, and ferment them to health-beneficial compounds such as short-chain fatty acids. Not every microbe can degrade all fibres. Some microbes are real specialists, able to degrade only one type of fibre, whereas others are generalists, able to degrade and grow on multiple dietary fibres.

With current needs for a circular economy, valorisation of side-streams of food industry into novel beneficial ingredients becomes more and more important. One type of such side-stream is e.g. potato fibre that is a left-over from potato starch isolation. Potato fibre consists primarily of the cell-walls of potato, which contain a mixture of dietary fibres, namely pectin, cellulose and hemi-cellulose. Recent research has shown that such insoluble fibre mixture could be a beneficial ingredient to be added to foods as a dietary fibre. However, it is not yet fully understood how potato fibre is degraded and fermented by gut microbiota. To elucidate the fermentability of potato fibre by gut microbiota, we need to track the degradation of the different dietary fibres within potato fibre. For this, we will monitor e.g. the sugar composition, linkage types and molecular weight distribution over fermentation time.



MSc projects:

- Structural characterisation of mixed fibres such as potato fibre by means of e.g. sequential fractionation, sugar composition, linkage analysis and molecular weight distribution
- *In vitro* fermentation of potato fibre by human gut microbiota and tracking the changes in chemical structure of mixed fibres over time using the techniques and approaches as listed above

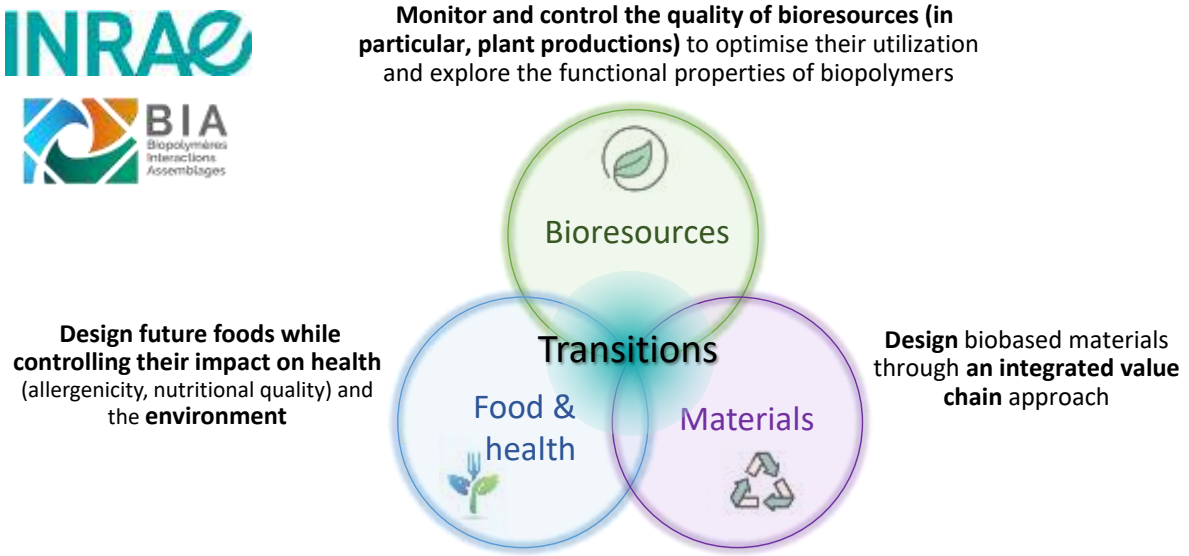
Topic 4.6 Biorefinery of fruit industrial pomaces

Specializations: *MFT* *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *E: Food Digestion and Health* *F: Gastronomy Science* *G: Dairy Science and Technology*	*Supervisors:*	*Carolina Pandeirada (WUR), Claire Berton-Carabin (INRAe)* *Topic suitable for: MSc*

Topic description: Fruit processing generates large amounts of by-products that represent a potential source of high-value molecules. Up to now valorization schemes are generally focused on one family of molecules. Here, using two types of apple pomaces (from two industrial sources), we propose to develop a scheme of biomass pre-treatments and biorefinery in order to design a multi-valorization process.

Project information: This is an initiative within the framework of our long-standing collaboration with dr. Claire Berton-Carabin (visiting associate professor at FPE and appointed at INRAE's research unit BIA - Biopolymers, Interactions, Assemblies; https://www6.angers-nantes.inrae.fr/bia_eng/) in Nantes, France). To strengthen the bonds between both institutes, the BIA unit offers some MFT thesis students to conduct their MSc thesis **in Nantes (FRANCE)**.

Practical information: The whole thesis **project will take place in Nantes (France)** from the start. Except for the examination, which will take place either at FCH in Wageningen or online. A dedicated department will help the student with housing, opening a bank account, and other practical arrangements in Nantes. In addition, the student will receive an allowance of 600 euros/month to cover additional costs.



An overview of the research focus of the BIA unit (Nantes, France) of INRAE, the French National Research Institute for Agriculture, Food and Environment.

Part 5 Biomass & Enzymology

The theme 'Enzymes and Biorefinery' studies the changes in carbohydrates and of lignin during plant biomass conversion processes. 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 theme.

We study mainly conversion of grasses, of which the plant cell walls are majorly composed of cellulose, hemicellulosic arabino-glucurono-xylan and of lignin. These three polymers contribute to the plant cell wall architecture, which influences physical characteristics like toughness (and degradability), and water binding capacity. A better understanding of the chemical fine-structure 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.

The aim of this theme is to (i) set up analysis for quantification and characterization of lignin; (ii) monitor changes in lignin and plant carbohydrate levels and composition during pretreatment, fungal growth, enzymatic processes, and during animal digestion; (iii) understand mode-of-action of hydrolytic and oxidative carbohydrate and lignin degrading enzymes and their effect on the chemical fine structure of natural substrates.

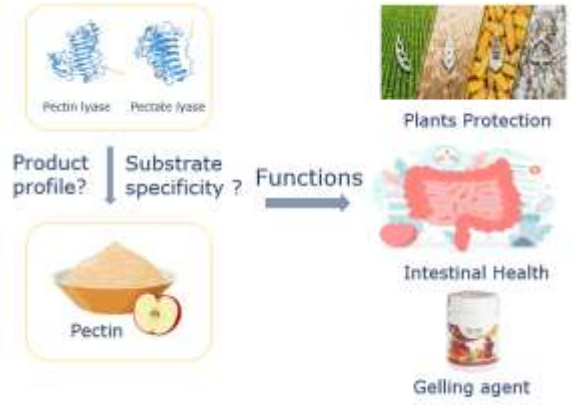
For the lignin analysis, we have set-up a new method via pyrolysis-GC-MS, making use of a mildly extracted ^{13}C-lignin isolate from wheat straw as internal standard. This method allows us to specifically quantify residual lignin content *in situ*, while simultaneously providing structural insights. Further lignin characterisation via NMR (HSQC) has been set up. 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 biorefinery, 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 laccases and the new lytic polysaccharide monooxygenases, are studied.

Topic 5.1 Production and characterization of pectin-active lyases



Specializations: *BFT* *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Supervisors: Nan Zhang Peicheng Sun Mirjam Kabel* *Topic suitable for: BSc & MSc*

Pectin comprises a group of complex and closely associated polysaccharides present mainly in the primary cell wall of plants. In the plant cell wall, pectin provides rigidity and texture, plays a role in water holding capacity, and is a key barrier for plant pathogenic attack and wounding. Extracted pectin has been widely used in pharmaceutical, dental and cosmetic industries. In addition, pectin (or partially modified pectin) is believed to enhance intestinal health and can be used as a prebiotic ingredient. Pectic oligosaccharides (POS) are derived from polymeric pectin and have been shown to have many functions such as prebiotics.



POS can be obtained by depolymerization of extracted pectin-fractions by enzymatic methods which are generally very specific. Currently, little is known about lyase of fungal origin, especially rhamnogalacturonan lyases (RGLs), including their production and purification, substrate specificity, regio-selectivity, mechanism of action and conditions of action. The application of novel pectin-active lyase to the production of POS may be able to create newly special structures, thus giving them new desirable properties. Our study might not only open opportunities for tailoring pectin to improve their bio- and physicochemical properties, but also might further contribute to crop protection and food functionality.

In this project, students will be involved in the process of the production and purification of lyases and learn how to determine their catalytic performance by characterising their effects on a range of pectic substrates. In the course of this project, you will be exposed to various protein purification techniques and methods of biochemical analysis, experiencing the fascination of enzymology.

MSc/BSc projects:

- Production and purification of fungal pectin-active lyases
- Characterise the biochemical properties of pectin-active lyases, such as their substrate specificity and regio-selectivity

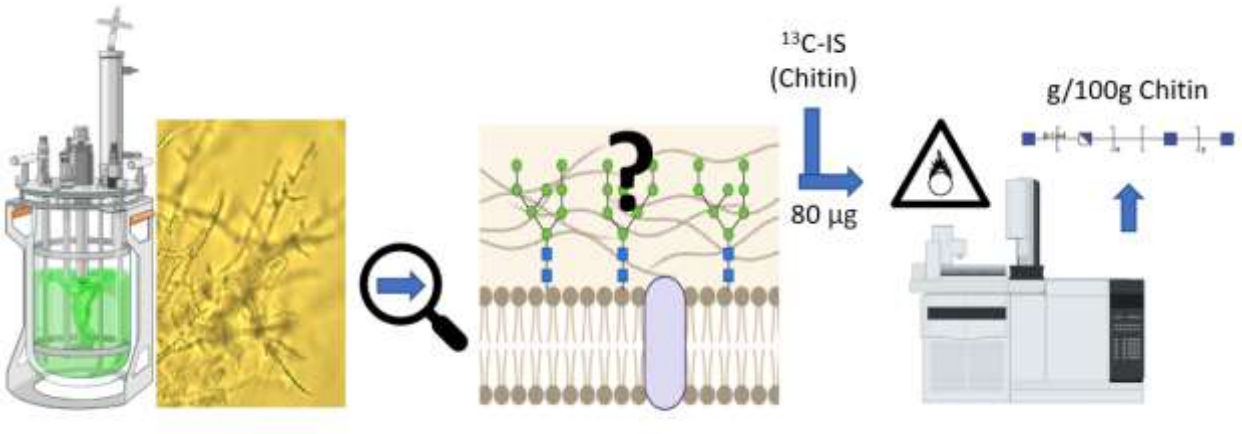
Techniques to be used: Chromatographic purification techniques, HPSEC, HPAEC, MALDI-TOF-MS, UPLC-MS

Topic 5.2 Quest for fungi that convert fiber-rich side-streams in animal-free food protein

Specialization: *A: Product Design and Ingredient Functionality* *C: Food Fermentation and biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Supervisors:* *Cas Geerits* *Mirjam Kabel* *Topic suitable for: MSc*

The global demand for protein has experienced a significant upswing over recent decades, which cannot be fulfilled by animal-derived protein sources alone. Moreover, animal protein does not align with vegan and vegetarian diets, while also being considered unsustainable. An animal free, cost-effective and locally sustainable, alternative is mycoprotein, a fungal based fermentation of carbohydrate-rich feedstocks into protein-rich products. The ultimate goal of this research is to develop a methodology to produce protein from fiber-rich food and agro-industrial side-streams, leading to a sustainable and scalable process for animal-free protein-rich food.

Fungal cell walls have a complex architecture, built from various glucans, chitin and glycoproteins. Standardized methods are often not applicable to fungal biomass. Analysis of these complex carbohydrates is a laborious process requiring the analyte to be solubilized before analysis. Chitin is relatively stable and requires harsh hydrolysis conditions to solubilize, during which structural features like degree of acetylation/crystallinity are lost. In this project Pyrolysis-GC-MS is investigated for the quantification of chitin. In order to quantify the amount of chitin present in fungal biomass, an internal standard is needed. We aim to use isotopic labeled ^{13}C-chitin as internal standard.



MSc project:
Investigate the isolation and analysis of chitin from fungal biomass using Pyrolysis-GC-MS

Part 6 Food lipids

The term 'Lipids' refers to a wide class of molecules, the main ones being fatty acids, glycerolipids, glycerophospholipids, sphingolipids, and sterols. They contribute to the flavour, texture, and nutritional value of food. Lipid oxidation is an important challenge for the food industry as it reduces nutritional value and generates off-flavour. Better understanding the mechanisms behind lipid oxidation will help developing innovative ways to prevent it.

The incorporation of polyunsaturated fatty acids in food formulations could be of great interest to promote health. These polyunsaturated fatty acids are involved in the regulation of inflammation and have shown benefits for cardiovascular and cerebral functions. However, these polyunsaturated fatty acids are also very sensitive to lipid oxidation, generating unpleasant off-flavours and reducing the nutritional value of these compounds.

Lipid oxidation in food is a complex process, that could lead to the formation of hundreds of different molecules. Moreover, the type of food matrix and the presence of other components (proteins, antioxidants, metals) could influence the process, making it even more complex. In order to prevent the generation of off-flavours and the loss of nutritional value of food due to lipid oxidation, it is important to better characterize these mechanisms.

The aim of this theme is to (i) set up analytical tools for characterization and quantification of lipid oxidation products (radicals, hydroperoxides, oxylipins, volatiles), (ii) monitor lipid oxidation in various types of food or food ingredients during storage and processing, (iii) understand the interaction with other food components, such as metals, proteins, and antioxidants, (iv) develop innovative and consumer-friendly methods to control lipid oxidation in food.

To this end, multiple analytical tools are used including Nuclear Magnetic Resonance (NMR) spectroscopy, Electron Spin Resonance (ESR) spectroscopy and various chromatographic and mass spectrometric methods.

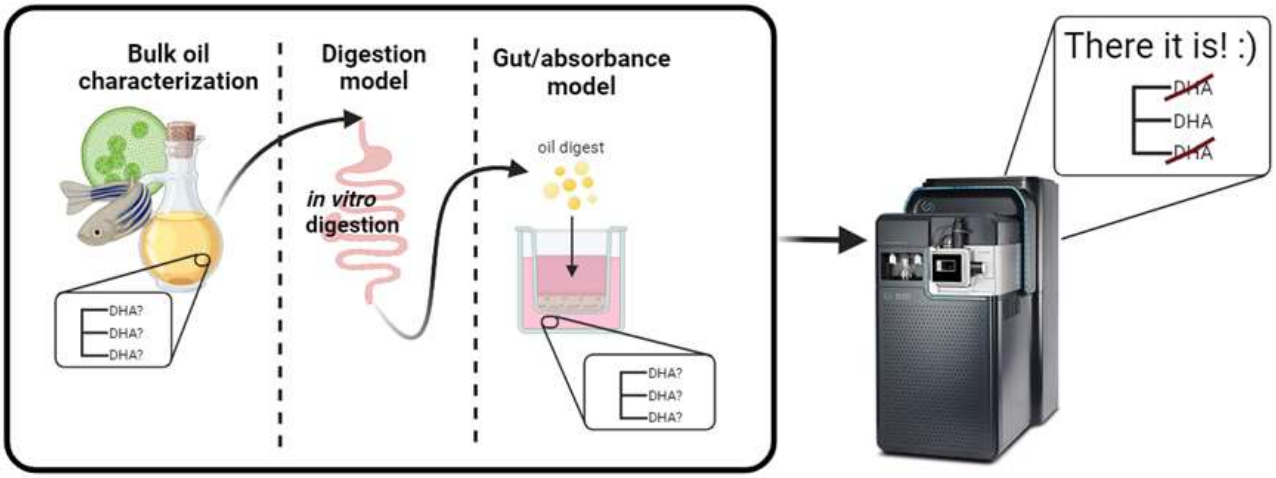
Topic 6.1 Where is the DHA? Using cyclic ion mobility MS to identify TAG isomers in bulk, digested, and absorbed oil



Specializations: *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *F: Gastronomy Science* *MBT-B: Food Biotechnology*	*Supervisors: Daniëlle Wessels Carlo de Bruin Marie Hennebelle* *Topic suitable for: MSc*

Long-chain (LC) omega-3 polyunsaturated fatty acids (PUFAs) are of great interest for their potential health benefits. Omega-3 is difficult to digest and it is even more difficult for omega-3 to reach the right body part. Docosahexaenoic acid (DHA) is the most important omega-3 fatty acid. Research has shown that the sn-position of DHA on the glycerol backbone in a triglyceride (TAG) is highly important for its metabolic fate. For example: if DHA is bound to the sn-2 position of a TAG, it is more likely to reach the brain than when it would be on the sn-1 position. Despite the constant developments in analytical strategies, none of current available techniques (e.g. LC-MS) are able to fully characterize sn-positional TAG isomers. Ion mobility spectrometry (IMS) is an upcoming analytical technique for isomer separation that involves gas phase separation of ions based on their mobility. Recently, a novel type of IMS has become available to our laboratory, named cyclic ion mobility spectrometry (cIMS). The cIMS is a promising state-of-the-art analytical technique for tackling current knowledge gaps and explore new boundaries in the characterization of isomeric lipids present in food products.

In this thesis project, we will investigate different omega-3-rich oils (fish and algae) and determine TAG isomers in the samples using cIMS-MS. Next to the pure oils, we will mimic digestion using INFOGEST and absorption using caco-2 cell models to see if the sn-position changes upon digestion and absorption.



MSc project:

- Determine the sn-position of DHA/EPA/other FA using cyclic ion mobility mass spectrometry in omega-3-rich algae and fish oil at different stages of digestion/absorption.

Techniques to be used: Cyclic ion mobility mass spectrometry, INFOGEST in vitro digestion, cell culture

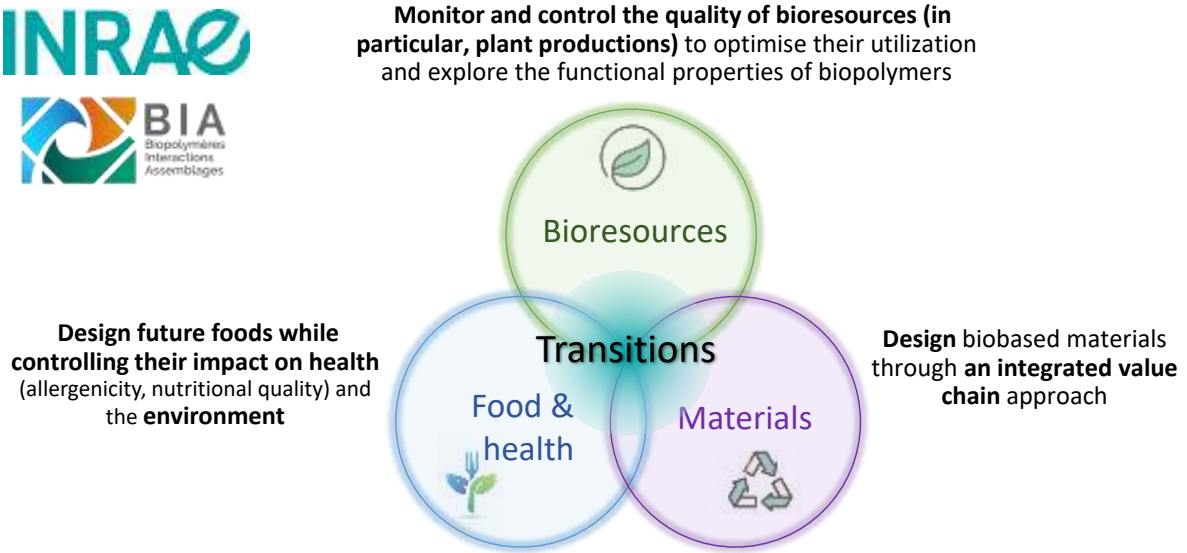
Topic 6.2 Preparation and functional properties of pea and faba bean protein fractions

Specializations: *A: Product Design and Ingredient Functionality* *C: Food Fermentation and Biotechnology* *E: Food Digestion and Health* *F: Gastronomy Science* *G: Dairy Science and Technology*	*Supervisors:* *Marie Hennebelle (WUR), Claire Berton-Carabin (INRAe)* *Topic suitable for: MSc*

Topic description: Plant proteins currently face increasing interest for use as food emulsifiers. In an ongoing project in our lab, we focus on pea and faba bean protein ingredients from various cultivars and aim at unveiling the interplay between the cultivar and inherent seed composition, the applied process to yield protein ingredients, and the ability of the ingredients to physically and oxidatively stabilize emulsions. In the present sub-topic, we aim to apply a new wet fractionation process and understand the potential consequences on the ingredient composition and properties.

Project information: This is an initiative within the framework of our long-standing collaboration with dr. Claire Berton-Carabin (visiting associate professor at FPE and appointed at INRAE's research unit BIA - Biopolymers, Interactions, Assemblies; https://www6.angers-nantes.inrae.fr/bia_eng/) in Nantes, France). To strengthen the bonds between both institutes, the BIA unit offers some MFT thesis students to conduct their MSc thesis ***in Nantes (FRANCE)***.

Practical information: The whole thesis **project will take place in Nantes (France)** from the start. Except for the examination, which will take place either at FCH in Wageningen or online. A dedicated department will help the student with housing, opening a bank account, and other practical arrangements in Nantes. In addition, the student will receive an allowance of 600 euros/month to cover additional costs



An overview of the research focus of the BIA unit (Nantes, France) of INRAE, the French National Research Institute for Agriculture, Food and Environment.

Part 7 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 plant-based meat alternatives. 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 theme of FCH, we aim to unravel the complex reactions involved in the formation of process flavors.

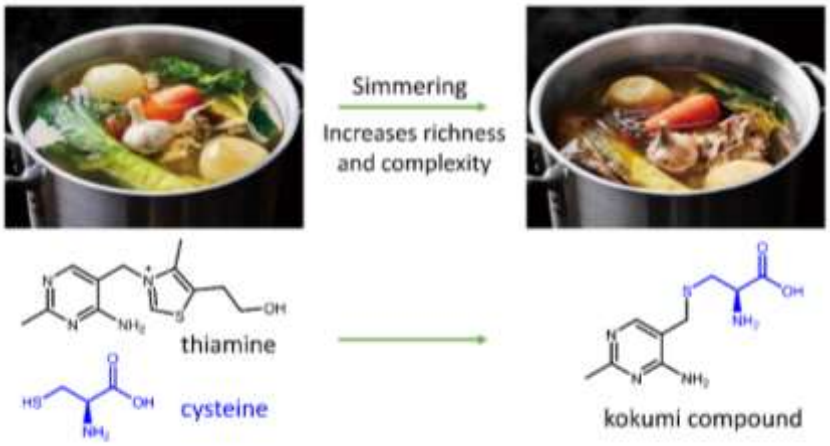
In addition to tastants and odorants, flavor-enhancing and flavor-modifying compounds may be formed during food processing and preparation. Such compounds have no inherent flavor, but may alter the perception of taste and smell. Therefore, these molecules can be interesting functional food ingredients, e.g. to enable salt or calorie-reduction without loss of flavor intensity. Within the Flavor Chemistry theme 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.

To work on the above-described aims, we use various techniques, such as UHPLC-MS, preparative chromatography, ion-mobility-MS, GC-MS, NMR and computational chemistry.

Topic 7.1 Taste-enhancing compounds for next-generation meat replacers

Specialization: *BFT* *A: Product Design and Ingredient Functionality* *F: Gastronomy Science*	*Supervisor:* *Angelina Hopf* *Roelant Hilgers* *Topic suitable for: BSc & MSc*

Plant-based meat alternatives play an important role in the protein-transition, by providing meat enthusiasts the opportunity to consume plant-based products that mimic the texture and flavor of meat. 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, there are kokumi compounds that are formed through heat-induced reactions, e.g. during simmering of a soup or stew and during roasting of meat (see example below).
Such kokumi compounds are not commercially available in pure form, which hampers in-depth sensory evaluations. In this project, we aim to investigate novel routes for the formation of thiamine-derived kokumi compounds, develop methods to purify (food-grade) kokumi compounds, and evaluate their suitability as flavor ingredients in next-generation meat replacers.



Thesis project:

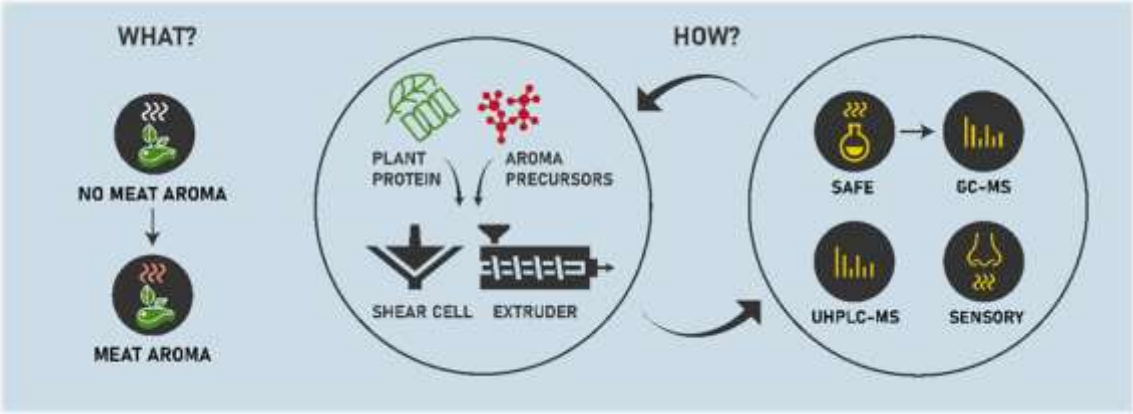
- Develop novel synthesis and purification methods for thiamine-derived kokumi compounds

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

Topic 7.2 Meat aroma generation in plant-based meat analogues using thermo-mechanical processing


Specialization: *BFT* *A: Product Design and Ingredient Functionality* *F: Gastronomy Science*	*Supervisor: Mirjam Hemelaar Roelant Hilgers* *Topic suitable for: BSc & MSc*

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 texturization of plant-based proteins. In contrast, it remains challenging to satisfactorily mimic the typical flavor of meat. 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. In this project, 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 a stable authentic meat aroma during thermomechanical processing of plant proteins. One of the first steps in this project is to define the current 'flavor-gap' between meat and plant-based meat analogues, i.e. which key meat aroma compounds are currently lacking in plant-based meat analogues. Then, we will try to generate these compounds during thermomechanical processing (in collaboration with the Laboratory of Food Process Engineering) by finding the right flavor precursors and optimizing processing conditions.



MSc project:

- Development and implement of a method to define the flavor-gap between meat and plant-based meat analogues

BSc project:

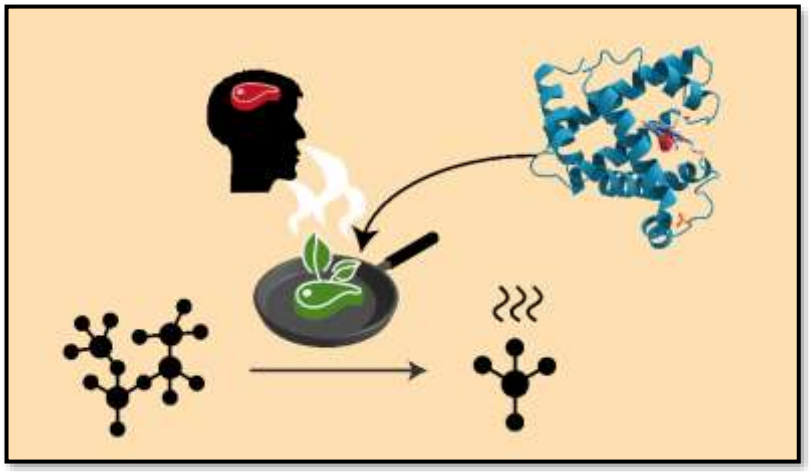
- Compare flavor extraction techniques (e.g. SAFE, SPME) to identify key aroma compounds in meat analogues using gas chromatography

Techniques to be used: Solvent-assisted flavor evaporation (SAFE), GC-MS, UHPLC-MS, potentially GC-O

Topic 7.3 Can heme-containing proteins catalyze meat aroma formation?

Specialization: *BFT* *A: Product Design and Ingredient Functionality* *F: Gastronomy Science*	*Supervisor: Roelant Hilgers* *Topic suitable for: MSc or BSc*

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. 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 e.g. plants and microalgae, 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 not inconceivable that heme-proteins somehow contribute to meat aroma generation during cooking, the underlying mechansism(s) are not understood yet. 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 (bovine) 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

Part 8 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. These assignments are used in the course Food Chemistry since 2000.

- **Online Problem Based Learning (group work**): by using tools such as Google Docs, Blackboard, 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.

- **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 8.1 Designing (digital) learning activities within the field of food chemistry

Specialization: *An interest in educational theories is required*	*Supervisors:*	*Bake de Rink*	*Julia Diederen*

Topic suitable 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 8.2 Find the answer to the food chemistry-related question that is already bugging you for long

Specialization: *BFT*	*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: Bake.derink@wur.nl / Julia.Diederen@wur.nl