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

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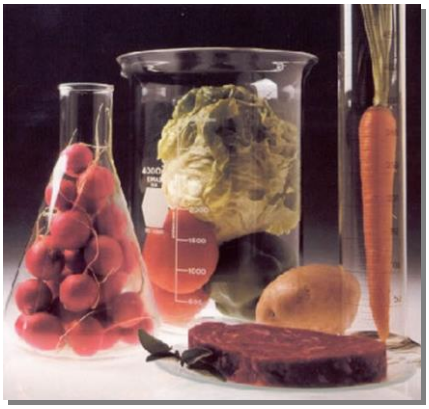
General information about a thesis research project at the Laboratory of Food Chemistry

Purpose of a thesis research project



Your thesis research project is an important part of your study. In the past years you have gained knowledge in food science with the aid of teachers and supervisors during lectures, laboratory classes, computer classes, etc. You learned obtaining information by literature searches, internet searches, performing experiments, evaluating your results and writing reports and/or giving presentations. Also, you have started to a certain extent to develop a scientific way of reasoning on how to solve problems. *The purpose of a thesis project is to further develop this skill to analyse and solve a problem in a scientific way.* In addition, a thesis project also perfectly serves the objective of obtaining knowledge/insight in a specific area of research.

Bachelor thesis
Bachelor topics comprise a desktop project with emphasis on data interpretation and report writing, which are important skills for the Master phase of your study. The data will be gather by means of literature study and data gathered within the laboratory by the thesis supervisor. At the end of the thesis you design an extended research set-up. You will work in a small thesis ring, in which you discuss parts of you report, to learn from other students, which will come together multiple times. You will have multiple workshops related to for example data management, literature search, writing skills, presentation skills and research design skills.

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 come together once every two weeks, to improve your writing skills.

A thesis research project at the Laboratory of Food Chemistry
After you have chosen a research topic, you will start-up the project together with your supervisor. The supervisor is usually a researcher from our Laboratory.

A thesis research project in Food Chemistry is related to a specific (bio)chemical food product or biomass conversion issue, which on its turn is related to an industrial application. In this way we do not solve an industrial problem, as this is the task of our graduates once they work in industry. Instead, we aim at understanding the mechanisms behind the problems and we aim to approach this in an academic way. With this knowledge/insight students are enabled to address (yet unknown) future problems, once they are graduated.

Within the Laboratory of Food Chemistry we focus our research on a limited number of topics. By doing so we are able to maintain our internationally well recognized position. This is also of benefit for students, as on the one hand their research project is well embedded and on the other hand it is a good reference regarding future employment. As a consequence the number of areas of research possible for thesis projects is somewhat restricted. In addition, to guarantee an optimal supervision, it is not always possible to choose the subject of your interest. This can be due to the fact that other students have already chosen the subject and for optimal supervision we want to restrict the number of students per supervisor.

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

- Part 1: Enzymatic or chemical reactions in food (raw) materials
- Part 2: Health aspects of food
- Part 3: New and/or improved ingredients for food
- Part 4: Educational development

Within these topics we deal with several foods and food raw materials such as dairy products, cereals, fruits, vegetables, legumes, beverages (coffee, tea, beer), etc.

Choosing a thesis research topic
First, formulate for yourself what specific knowledge/skills you want to learn during the thesis project. If you are e.g. interested in enzymology use this aspect for making your choice.
Second, is the background of the subject motivating you? If you do not like the background of the subject it will decrease the chance of success. Be aware that although it might be interesting to work on an industrial problem or product, it is the underlying way of performing research that is most important. By doing this you are, as stated above, enabled to address any industrial future problem. In order to acquire a solid background in food chemistry this can usually be trained better if one works with specific components of food rather than with the complete food product itself.

On the following pages you can find the description of the research topics. For each topic several possible more specified thesis projects are described.

During the open house, you can visit our laboratory and talk with the supervisors of the topics that you are interested in.

When you have made your choices, please indicate your choices by giving the number of the research topic.

We are sure you will be able to find a topic that fits you. Many students have preceded you and enjoyed their thesis projects.

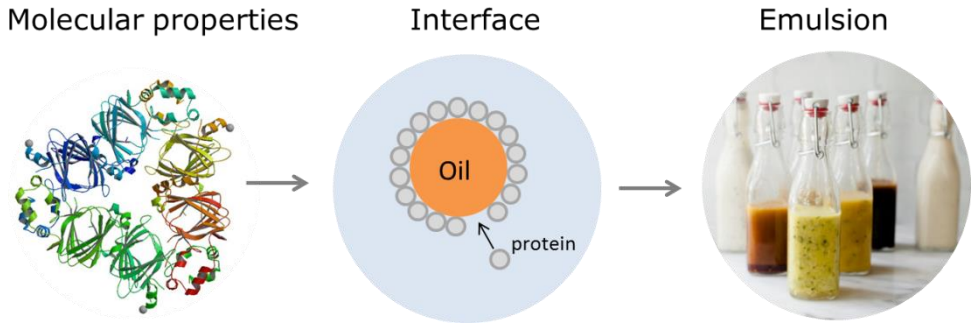
the staff of Food Chemistry

Part 1 Enzymatic or chemical reactions in food (raw) materials

Topic 1.1 Emulsion and foam properties of plant proteins

There is a strong interest to increase the use of plant-derived protein in food products. Especially oilseed and leguminous proteins (e.g. rapeseed, soy) have received a lot of attention, because of their nutritional and functional properties. Plant proteins can be used to form and stabilise emulsions and foams.

Typically, a trial-and-error approach is used in studies on emulsion and foam properties, resulting in scattered observations. As a result, sustainable novel protein sources are frequently rejected unnecessarily. To effectively screen the applicability of novel proteins, protein molecular properties should be quantitatively correlated to the emulsion and foam properties. The efficiency of different proteins to stabilise emulsions or foams is hypothesised to depend on the maximal adsorbed amount as well as on the adsorption kinetics. Both these parameters are linked to the quantifiable properties radius, charge, and hydrophobicity of the proteins.



BSc projects:

- Literature review of articles on emulsion and foam properties of plant protein (mixtures).
- Study by analysis of available data (provided / literature) the effect of system conditions on the molecular structure of multimeric proteins.
- Study by analysis of available data (provided / literature) the emulsion and foam properties of binary mixtures of globular proteins

Possible MSc projects:
In this project, you will study how the molecular properties of plant protein (mixtures) correlate to the interfacial, emulsion and foam properties. The project will focus on one or more of the following topics:

- Chemical characterization of protein (mixtures) from purified ingredients (e.g. SEC, CD, DSC, hydrophobicity and charge).
- Study the effect of system conditions on the molecular, interfacial, emulsifying, and foaming properties of different plant protein (mixtures) (e.g. Automated Drop Tensiometer, FoamScan).
- Develop a quantitative model with which emulsion and foam properties of protein mixtures are predicted.

Supervisors:  *Maud Meijers,*  *Peter Wierenga*

Topic 1.2 Free radical intermediates produced during primary lipid oxidation in O/W food emulsions

Food emulsions (O/W) such as mayonnaise and salad dressing are very prone to deterioration caused by lipid oxidation. The dispersed oil droplets contain unsaturated fatty acid chains that can be readily oxidized via diverse mechanisms. The first pathway of this oxidation is a free radical chain reaction, leading to hydroperoxide formation. Degradation of these hydroperoxides leads to the formation of so-called secondary oxidation products (e.g. aldehydes, epoxides), which are responsible for the rancidity and off-flavor of O/W food emulsion products. The mechanistical pathways of lipid oxidation have been partly unraveled in bulk oils. However, in food emulsions, both chemical and colloidal effects come into play. Unravelling of these mechanisms is hampered by lack of knowledge on unstable radical intermediates.

This project will focus on the initial stage of primary oxidation and the transition stage between primary and secondary oxidation. Here we will aim to identify and quantify the radical intermediates by Electron Spin Resonance (ESR). This technique allows for the detection of stable radicals. In lipid oxidation, formed radicals are very reactive and short-lived, making it impossible to analyze them as such. To tackle this problem, so called spin-traps can be used. The emulsion matrix adds significant complexity to the applicability of a spin-trap, which needs to be considered when selecting the right spintrap. Ultimately, the ESR-method will be applied in shelf-life tests, where we want to be able to resolve the formation of specific radicals in time.

ESR-facilities are located in the Helix building at the WUR-campus. Samples will be generated in collaboration with Unilever R&D Vlaardingen.

Possible BSc project:

- Analysis of available ESR data of free radical intermediates in food emulsions by ESR and investigation of their respective identification by literature and data research

Possible MSc projects:

- Identification and quantification of free radical intermediates produced during primary lipid oxidation in O/W food emulsions by ESR

Supervisors: Donny.Merkx@unilever.com, Marie Hennebelle, Peter Wierenga

Topic 1.3 New analytical techniques for research on food

During the last years a lot of new techniques and technologies were introduced which allows the food chemist to monitor reactions which take place in agricultural products during storage and processing. Nowadays it is possible to monitor chemical reactions in food products which was impossible only a few years ago. The discipline of proteomics would not have evolved so quickly without the development of advanced mass spectrometry techniques. Therefore, it is now possible to determine the structure of complex mixtures of bio molecules.

The techniques which are available and used at the Laboratory of Food Chemistry are described well. But for the introduction of these new techniques for the monitoring of different components it is sometimes necessary to carry out methodology research. For example: the quantification of components when new HPLC detectors are used, the better understanding of the fragmentation reactions which take place during the ionization of oligosaccharides in the LC-MS or the improvement of HPLC separation of complex mixtures of polysaccharides. The student should have a good perception of analytical techniques.

Possible MSc projects:

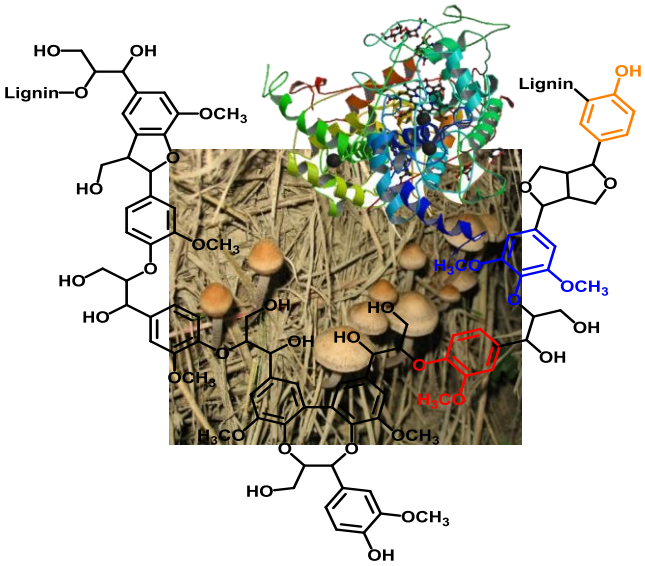
- Application of LC-MS, MALDI-TOF MS, HPLC.

Supervisors: Edwin Bakx, Henk Schols

Topic 1.4 Enabling the mapping of lignin: fungal modification and degradation

Biological conversion of lignin, or lignin-rich raw materials, like plant biomass, plays a role during the cultivation of mushrooms. Vice versa, fungi are an interesting tool to modify, degrade or convert lignin. The exact effect of (various) fungi on the lignin structure is unknown. In addition, it is known that certain fungal species produce enzymes, which may alter or degrade lignin structures – however, underlying mechanisms need further elucidation.

To accommodate development of new ingredients from lignin and improve feed digestibility, the relation between the lignin structure and possible enzyme or fungal effects on this structure needs to be fully understood. Hereto, the chemical structure and quantification of lignin is required for which isolation of (various) lignin fractions, a highly challenging task, is aimed at.



In this highly analytical project multiple techniques will be employed to comprehensively characterise lignin and map its degradation by fungi. Analytical techniques include, amongst others, pyrolysis coupled to gas chromatography with mass spectrometric detection (py-GC/MS), nuclear magnetic resonance (NMR) and size-exclusion chromatography.

BSc thesis projects:

- Investigate by analysis of available data (provided / literature) for several fungal treated biomass samples the low molecular weight lignin-derived degradation products

MSc thesis projects:

- Comprehensive analysis of fungal grown plant biomass samples (examples of relevant analytics are py-GC/MS, NMR, HPSEC or UHPLC-MS).
- Mild isolation and characterisation of lignin from various raw materials, including mapping lignin/ phenolic compounds-like structures in the various fractions obtained.
- Method development for quantitative lignin analysis with py-GC/MS.

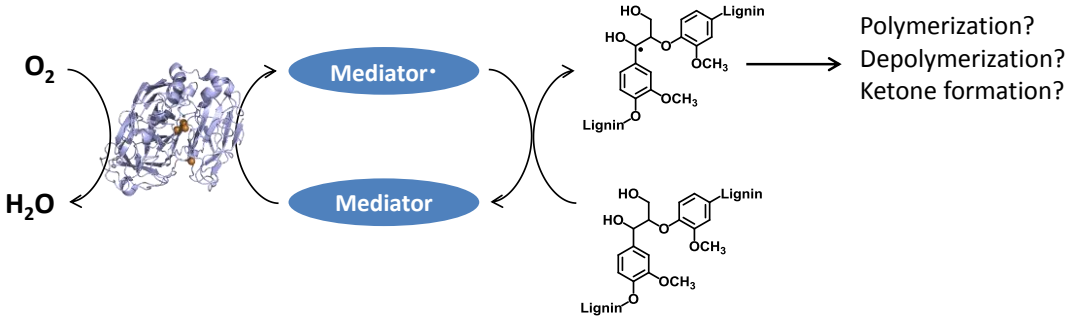
Supervisors:

Gijs van Erven

Mirjam Kabel

Topic 1.5 Can laccase-mediator systems upgrade lignin: new natural ingredients?

Lignin is a polymeric aromatic structure in plant cell walls, and is one of the most abundant polymeric materials in nature. Its insolubility and recalcitrance to enzymatic breakdown make lignin breakdown one of the largest challenges in biorefinery.
Although challenging, enzymatic breakdown of lignin may open up new ways towards natural ingredients for food & feed products or as natural chemicals, e.g. as anti-oxidants or anti-microbials.
Laccases are among the few enzymes that show activity towards lignin. These enzymes couple the reduction of O_2 to the one-electron oxidation of aromatic substrates. For the oxidation of the majority of the lignin subunits, it is necessary to combine laccase with a mediator (a small molecule that acts as electron carrier between enzyme and substrate). The initial oxidation of lignin by laccase-mediator systems has been suggested to result in a competition between lignin depolymerization, ketone formation at the C_α atoms, or lignin polymerization. Currently, it is poorly understood how this competition is balanced.



Before laccase-mediator systems (LMS) can be applied to result in new natural ingredients, we need to be able to predict the effect of these systems on the substrate structure.
This project aims at understanding how laccase-mediators act on lignin and lignin-like structures. The focus is on the competition between polymerization, depolymerization and C_α oxidation of lignin, as initiated by laccase-mediator systems.

BSc thesis project:

- Investigate by analysis of available data (provided / literature) for several situations (e.g. different substrate structures, reaction conditions) how lignins and lignin model compounds are modified by laccase and LMS.

MSc thesis projects:

- Understand the effect of different LMS on isolated lignin (UHPLC-MS, SEC, NMR, other).
- Understand the influence of reaction conditions on incubations of lignin and lignin model compounds with LMS (UHPLC-MS, SEC, NMR, other).

Supervisors:

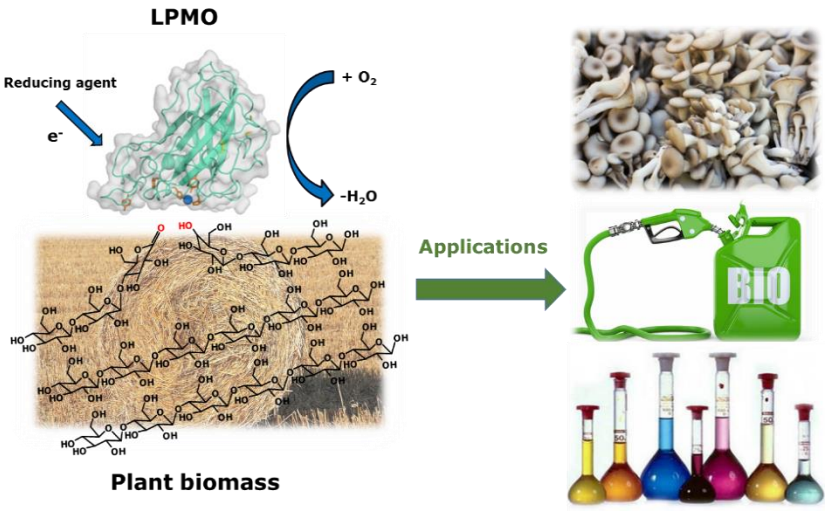

Roelant Hilgers


Mirjam Kabel


Jean-Paul Vincken

Topic 1.6 Oxidative cleavage of polysaccharides by LPMO enzymes - a new approach in biomass refinery

Fungi boost the deconstruction of lignocellulosic plant biomass via oxidation using lytic polysaccharide monooxygenases (LPMOs) acting in synergy with hydrolytic carbohydrate degrading enzymes. The future application of LPMOs is expected to contribute to ecologically friendly conversion of biomass into fuels and chemicals. Moreover, applications of LPMO-modified cellulose-based products may be envisaged within the food or material industry.



LPMOs represent a unique class of enzymes shown to oxidatively cleave crystalline cellulose and xylan associated cellulose in the presence of a reducing agent and oxygen.

In the last 5 years of various LPMOs the substrate specificity, regio-selectivity and reducing agent preference have been studied. Still, many aspects of many 'putative' LPMOs are unknown. This thesis project includes the research of new LPMOs, new methods to quantify their activity and synergy of LPMOs with hydrolytic enzymes.

BSc projects:

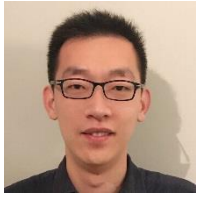
- Investigate the effect of carbohydrate binding modules on enzyme activity by analysis of available data (provided / literature).

MSc projects:

- Purify new LPMOs and characterise their substrate-specificity and regio-selectivity (e.g. SDS-PAGE, chromatographic purification techniques, HPAEC and MALDI-TOF-MS)
- Develop a method for quantitative analysis of C4-oxidised products (e.g. HPAEC, RP-UHPLC-MS/MS and MALDI-TOF-MS)
- Investigate the effect of cellulose crystallinity and/or lignin on the activity of LPMOs (e.g. HPAEC, X-ray spectroscopy and MALDI-TOF-MS)

Supervisors:

Peicheng Sun



Mirjam Kabel

Topic 1.7 Non-enzymatic browning as observed in RTD

Prevention of undesirable browning seems to be an issue in tea manufacturing, especially with ready-to-drink (RTD) green teas. This problem becomes evident during storage of in RTD green tea upon which non-enzymatic auto-browning undoubtedly leads to a quality defect. This phenomenon was reproduced in our laboratory under more controlled conditions. The chemical constituents responsible for the brown colour are still unknown. In order to mask the off-colour of RTD green tea infusions, manufacturers commonly use green polyethylene terephthalate bottles or non-transparent packaging. Important as it is, relatively little research has been devoted to such non-enzymatic auto-oxidation in aqueous systems in RTD drinks.

BSc projects:

- *Investigate several potential factors affect colour in RTD tea*

Possible MSc projects:

In this project, you will study by which mechanism the non-enzymatic oxidation of phenolics proceeds, and to identify the key factors (temperature, pH, or oxygen) influencing this process.

- *First, the changes in colour in RTD tea will be recorded at different temperatures, pH values, and oxygen levels, in order to systematically determine the key parameters behind colour change. A preliminary screening by UHPLC-MS will be carried out to determine which of the green tea phenolics disappear and are thus most likely to be involved in the browning process.*
- *Second, model systems will be used to verify the role of the key contributors to this non-enzymatic browning, and to establish which brown products are formed.*

Supervisors:

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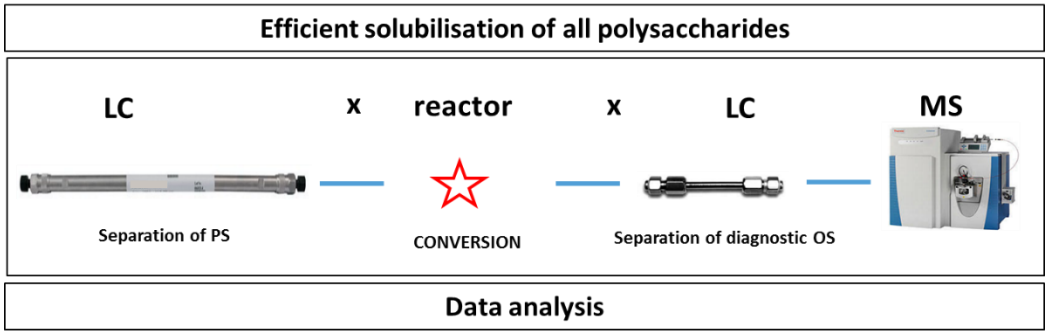
Jean-Paul Vincken

Topic 1.8 The analytical challenge: Polysaccharide sequencing

Polysaccharides are the main constituents of plant material determining the texture of fruit, vegetables and cereals. For this reason, polysaccharides have increased interest from the food and feed industry. For a more efficient utilisation and functionalisation of plant's polysaccharides, and the development of whole new food and feed products, we heavily rely on knowing the precise chemical structure of all polysaccharides present as relatively small structural differences within one polysaccharide may already result in huge differences in its behaviour. Unfortunately, such compositional knowledge on plant's polysaccharides is still very limited as their complexity hampers significantly their analysis. Sequencing of polysaccharides as is routine for proteins is not yet possible. Therefore, there is a strong need in novel strategies to characterize polysaccharides faster, more generic, more detailed and in a more integrated approach.

The novel approach consists of 1) efficient solubilisation of insoluble polysaccharides, 2) LC×reactor×LC separation, and 3) detection (identification, quantification) of these diagnostic oligosaccharides followed by data analysis.



BSc projects:

- *Investigate for several situations routes for degradation of polysaccharides to diagnostic oligosaccharides*
- *Investigate for several situations routes for efficient solubilisation of polysaccharides while maintaining their structural information.*

Possible MSc projects:

- Screening of various polysaccharide degradation protocols to obtain diagnostic oligosaccharides for suitability within an LC×reactor×LC approach.
- Development rapid LC separation method for diagnostic oligosaccharides, including MS detection.

Supervisors:  *Carolina Pandeirada*  *Yvonne Westphal*

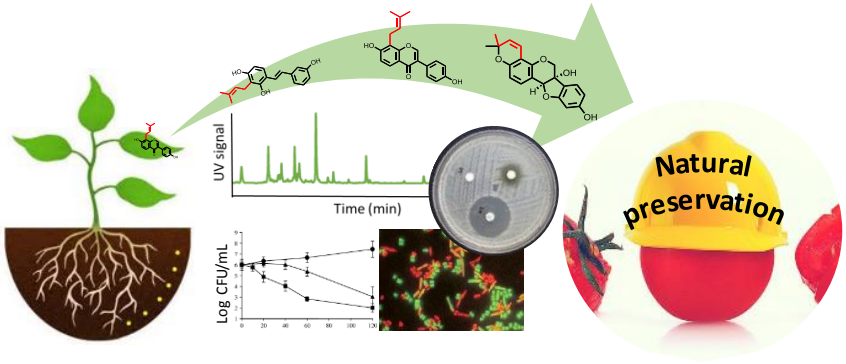
 *Henk Schols*

Part 2 Health aspects of food

Topic 2.1 Quantitative structure-activity relationships and mode of action of plant antimicrobials against food pathogen and spoilage microorganisms

As a result of consumer's demands for more natural and tasty foods, manufacturers are looking into novel ways of preserving foods without intensive processing (e.g. heating) or use of traditional synthetic antimicrobials (e.g. benzoates). An attractive way for achieving this is to make use of the weaponry offered by plants to combat microorganisms.

Prenylated (iso)flavonoids (i.e. those with a C5-substituent) are one of the main families of secondary metabolites produced by legumes as part of their defence system (e.g. upon bacterial infection). Some prenylated isoflavonoids have shown strong inhibitory activity towards human pathogens, including multidrug resistant strains. Nevertheless, this activity seem to be highly structure- and microbial-dependent. Furthermore, because plants produce a vast variety of defence secondary metabolites when stressed, it is expected that these metabolites can work synergistically by targeting different (bacterial) cellular processes. By using optimal combinations, smaller amounts of antimicrobials can potentially be used while achieving the same level of preservation.



To develop novel and effective antimicrobials from plant secondary metabolites it is necessary to fully understand these structure-activity relationships against a variety of food relevant microorganisms. Additionally, to develop preservation strategies based on synergizing combinations of natural antimicrobials it is essential to elucidate and understand their mode of action.

BSc project

- Investigate by analysis of available data (provided / literature) the potential of different families of plant secondary metabolites as food preservatives (potency, microbial specificity, mode of action, structure-activity relationships).
- Investigate by analysis of available data (provided / literature) techniques of testing the mode of action of plant antimicrobials.

MSc projects

In general, thesis projects will include both chemical and microbiological practical work (previous experience in microbiology is appreciated).

- Characterization and purification of antimicrobial plant metabolites by chromatographic techniques, such as UPLC-MS, FLASH and preparative HPLC.
- Elucidation of (quantitative) structure-activity relationships of (purified) plant antimicrobials against food relevant microorganisms (pathogenic and spoilage).
- Elucidation of the mode of action of the main plant antimicrobial compounds.
- Study the synergistic effects of plant antimicrobials against food relevant microorganisms.

Supervisors:

Carla Araya Cloutier

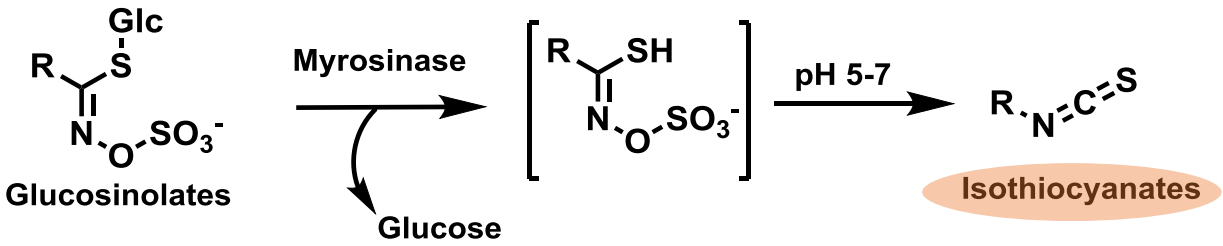
Jean-Paul Vincken

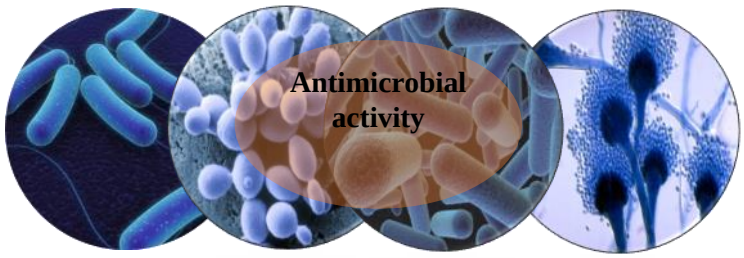
Topic 2.2 Antimicrobial Activity of Brassica Isothiocyanates and Their Mode of Action

There is a growing interest in finding novel antimicrobial agents because of the increasing consumer demand for more natural food and incidence of antibiotic resistance. Nonetheless, finding new potential antimicrobial agents against pathogenic Gram-negative bacteria is a challenge due to their specific feature, called efflux pump system. Previous research in Laboratory of Food Chemistry has shown that in the absence of efflux pump inhibitor, prenylated (iso)flavonoids do not show growth inhibition in Gram-negative bacteria, e.g. *Escherichia coli*.

Therefore, we are investigating another type of compounds, namely isothiocyanates. They are derived from glucosinolates, the major secondary metabolites in the plant family Brassicaceae, e.g. cabbage, mustard, and broccoli. We have found that they are antimicrobial active.

Beside against Gram-negative bacteria, we also extend the investigation to Gram-positive bacteria, e.g. *Bacillus cereus*. We have observed that isothiocayanates have the antimicrobial activity against both Gram-negative and Gram-positive bacteria. The antimicrobial activity of isothiocyanates is varied depending on the side chains (-R) as well as on the microorganisms.





BSc projects:

- Investigate by analysis of available data (provided / literature) for the microbial factors determining the microbial susceptibility towards isothiocyanates
- Investigate by analysis of available data (provided / literature) for the structural features and incubation condition of isothiocyanates determining their antimicrobial activity

Possible MSc thesis projects:

In general, these thesis projects will include both chemical and microbiological practical work.

- Elucidation of the quantitative structure-activity relationship (QSAR) of antimicrobial activity of different isothiocyanates against E. coli and Bacillus cereus.
- Elucidation of the mode of action of the main antimicrobial isothiocyanates against E. coli and B. cereus.

Supervisors:  *Silvia Andini,*  *Jean-Paul Vincken*

Topic 2.3 Natural Microbial control: Ways of elicitation to biosynthesize natural antimicrobials from legumes

The long-term challenge of the project "Natural Microbial Control" is to employ natural antimicrobials in food products to prevent spoilage, without risking the development antibiotic resistance against them. A way to achieve this is by making use of the weaponry offered by plants. Plants of various species are known to produce defence compounds (phytoalexins) when they are exposed to stress. Stresses are roughly divided into biotic stresses (elicitors), including infection by microbes or fragments thereof and abiotic stresses (elicitors), such as physical damage (wounding), exposure to UV irradiation and treatment with chemicals. Upon sensing these stresses, the stress signal is transferred from the site of infection further to downstream reactions by plant hormones that act as messengers. Part of this defence reactions includes the biosynthesis of antimicrobial compounds. Legumes is one of the largest plant families known to synthesize such compounds (Figure 1). To a very high extent, these compounds bear a prenyl-group (shown in yellow in Figure 1), which is thought to be the key player with respect to antimicrobial activity. In our laboratory, a malting protocol has been established in which germinated legume seeds are stressed by application of a food-grade fungus. The induction of phytoalexins has mainly been done by using Rhizopus spp. as a biotic stressor (elicitor). Using Rhizopus elicitation as a benchmark experiment, variations in the time of application of the fungus or in the type of microbe in general, have not been investigated systematically. Moreover, the effectiveness of combinations of biotic and abiotic elicitors on the amounts and diversity of induced antimicrobials have also not studied thoroughly.

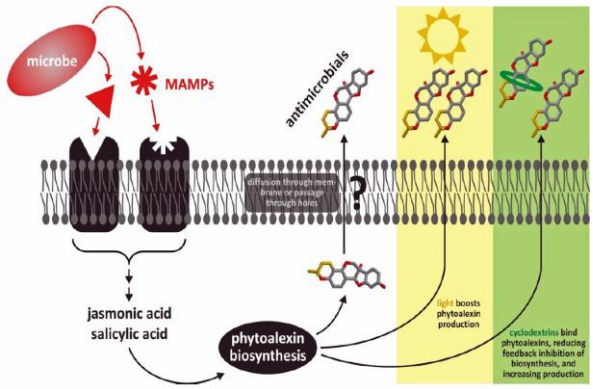


Figure 1. Factors potentially influencing the accumulation of phytoalexins in seedlings

Figure 2. Germinator used in micro-malting, normally used in the beer brewing industry for pilot-scale malting.

Another long-term goal of the project is to study and understand the antimicrobial properties of these compounds. Since antimicrobial activity has been found to be dependent on the overall configuration of the molecule, rather only on prenylation, systematic Structure-Activity Relationships, covering the large structural diversity of these prenylated compounds need to be established.

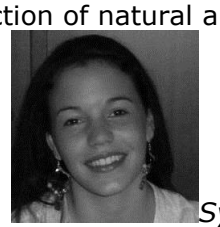
BSc projects

- Investigate and establish Quantitative-Structure Activity Relationships (QSAR) *in silico* of natural antimicrobials from legumes (given database) to determine the molecular features responsible for the antimicrobial activity.

MSc projects:

In general, these MSc thesis projects will include practical work mainly on chemical aspects but also some microbiology.

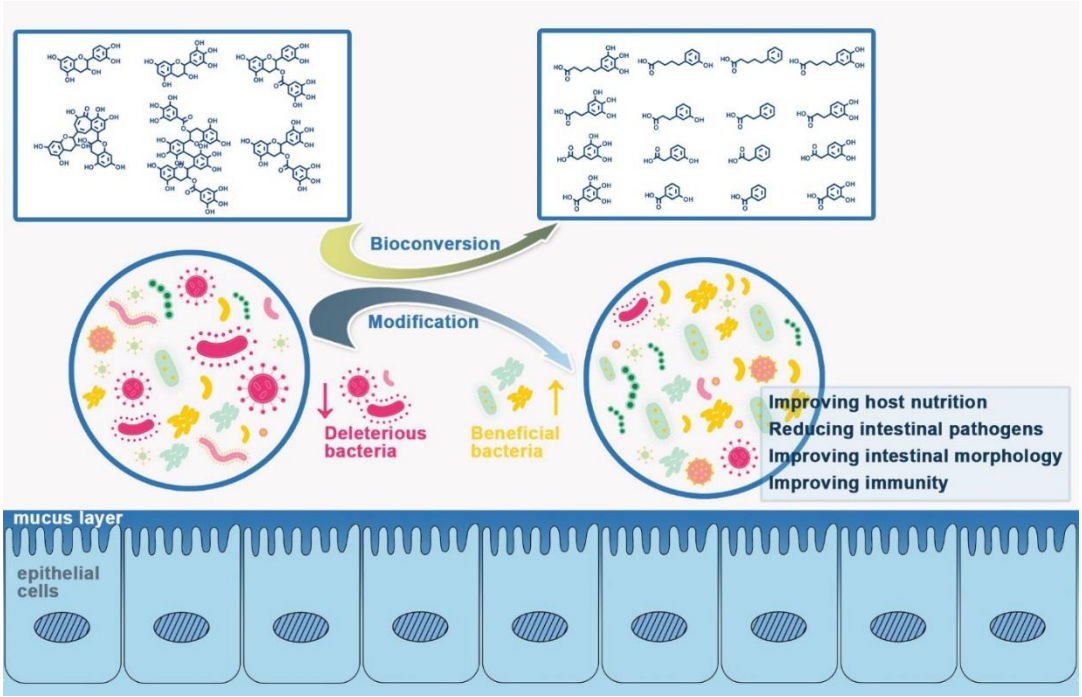
- Optimize the conditions of biotic elicitation and
- Combine the best biotic elicitation treatment with abiotic elicitors (eg. light or chemicals). Any of the above will be followed by extraction, fractionation, purification, and characterisation of phenolic compounds by (preparative) liquid chromatography (LC) and mass spectrometry (MS).
- Extrapolation of the findings to a variety of legume species to enlarge our collection of natural antimicrobials

Supervisors:  *Sylvia Kalli,*  *Jean-Paul Vincken*

Topic 2.4 Interactions between tea phenolics and gut microbiota

Tea phenolics have shown promising health benefits in epidemiological and human intervention studies. However, due to the low bioavailability, most of ingested tea phenolics retain to the colon where gut microbiota catabolize them into absorbable metabolites, mainly phenolic acids, which may be an important factor for promoting health. Simultaneously, composition and function of gut microbiota are modified by tea phenolics, consequently exert health benefits.

Thus, we hypothesize that tea phenolics, including green tea catechins and black tea polyphenols can be used as a new prebiotic material possessing a gut microbiota modulating effect. By co-culturing the tea phenolics and gut microbiota *in vitro*, the bioconversions of tea phenolics and temporal dynamics of gut microbiota will be studied with metabolomics and genomics approaches to evaluate the prebiotic-like effects of the tea phenolics.



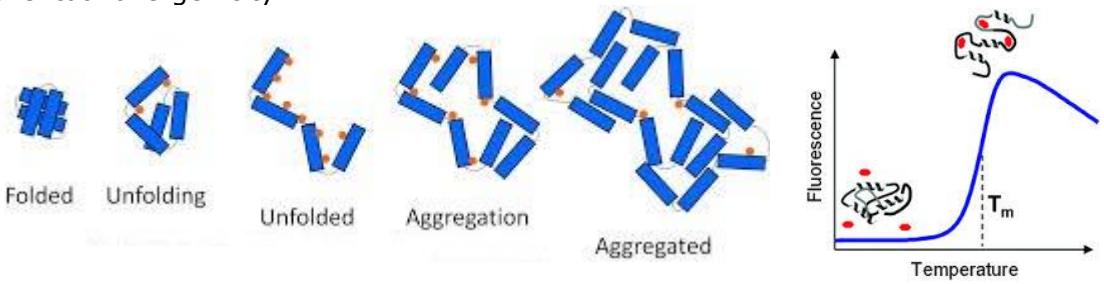
MSc project:
In this project, you will study the degradation pathway of tea phenolics when incubated with gut microbiota.

- The degradation pathway of the four primary catechins in green tea, including epicatechin, epigallocatechin, epicatechin gallate, epigallocatechin gallate.
- The degradation pathway of the green tea extracts, which contain a mix of diverse catechins.
- Whether different volunteers have different metabolic abilities on green tea catechins.

Supervisors:  *Zhibin Liu,*  *Jean-Paul Vincken*

Topic 2.5 Effects of processing of proteins on their immunogenicity: allergy as a model (dairy subject)

Food allergy develops in two phases: a sensitisation phase and an elicitation phase, the latter resulting in clinical symptoms of allergic disease. Sensitisation is the phase during which immunological priming to the inducing allergen occurs, leading to the evolution of aTh2-biased immune response and the production of IgE-antibody.
Skewing of adaptive immune response to a Th2-type phenotype and IgE-antibody production necessarily are preceded by an innate immune response to the allergen. Activation of receptors on cells of the innate arm of the immune system is crucial for the initiation of adaptive immune responses.
Processing changes the structural and chemical properties of proteins, and hence of allergens. Proteins denature, aggregate, bind to lipid structures, and undergo glycosylation and or glycation (Maillard reaction). These processing-related structural and chemical changes will influence how the immune system will respond, possibly leading to eventual allergenicity.



The aim of this project is to study the effect of processing-induced alterations in proteins (unfolding, fibril formation, aggregation, Maillardation) on the response that they provoke in (innate) immune cells, such as macrophages and dendritic cells.

Possible BSc and MSc projects:

- Characterisation of processing-induced structural alterations in (model) food proteins (fluorescence, circular dichroïsm, size, electrophoretic behaviour, etc.)
- Analysis of immune response to such alterations by exposing model cell cultures to the proteins by immunologic methods such as phagocytosis and cytokine production

Supervisors: Harry Wichers

Topic 2.6 Biological fortification of rice?

Rice contains a certain amount of metals, such as zinc, that are considered important from nutritional point of view. Especially in regions of need, supplementation of rice with metals has been considered to add to the daily intake of such nutrients. The regular supplementation is just addition of the required metal salts to the rice. However, it has also been observed that the content of essential minerals (specifically zinc) is influenced by the conditions under which the rice is grown.

In this project, you will study the way in which zinc is accumulated in rice grown under different conditions. The project will be in collaboration with the plant sciences group.

BSc projects:

- *A literature study on the accumulation of minerals, binding of zinc to proteins and other nutrients in rice and other plants will be performed.*

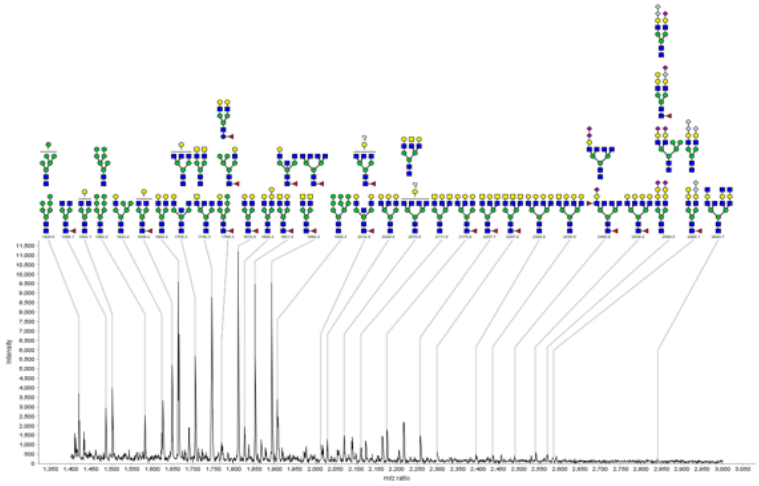
Possible MSc projects:
The aim is to study the way in which zinc is accumulated in rice. The questions are whether the zinc is specifically bound to proteins, or to for instance starch. For this several separations need to be performed after which the amount of zinc in each fraction needs to be determined.

- Purification and separation of soluble, insoluble proteins and other compound classes.
- Test and develop analyses to determine the zinc content. (currently we can send samples away for analysis, but it would be convenient to have an alternative method available 'in-house')
- Analysis of the proteins and other compounds in each fraction, and quantification of the zinc.
- Analytical methods will involve SDS-PAGE, capillary electrophoresis, and liquid-chromatography –mass spectrometry.

Supervisors:  *Rene Kuijpers,*  *Peter Wierenga*

Topic 2.7 N-Glycans in human milk (dairy subject)

Milk is the first source of nutrition for newborns. Newborns have an immature immune system and milk contains many biofunctional components, such as oligosaccharides and proteins, that are known to be involved in the development of the immune system. Proteins play a pivotal role in protecting the gut mucosa against pathogens and are also known to play an important role in the protection of the mammary gland. Since the proteins in milk are highly glycosylated, glycans are considered to be a major contributor to this immune system development.



This project will focus on the release of glyco-tails from the proteins. Recently, there is a method developed in our lab for the glycation of human milk proteins which needs validation. After this the method will be used to analyse the glycosylation patterns of proteins present in milk from different mothers during lactation.

BSc projects:

- Investigate by analysis of available data (provided / literature) the role of N-glycans during protein digestion using proteomics techniques

MSc project:

- Labelling and quantification of N-glycans, and to obtain a overview of all N-glycans present in human milk.

Supervisors:  *Moheb Elwakiel;*  *Henk Schols*

Topic 2.8 Toward controlled steering of microbiota and immunity in infants by non-digestible carbohydrates

Bacteria colonizing the infant mucosa guide the development of a balanced immune system and also support maturation of the gut-barrier. Mother-milk has been considered the golden standard for guiding this colonization. Mother-milk contains energy sources for microbiota and also supports immune function directly. For those infants where mother-milk is not a feasible option, cow-milk formulas supplemented with non-digestible carbohydrates (NDC) are used. An important function of these NDCs is a preferred support of Th1-responses responsible for fighting infections. However, recently it has been found that not all NDCs currently applied support Th1-responses and that induced responses are dependent on the composition of NDCs.

The goal of this project is the design of tailored NDC-supplements for healthy and disease-prone infants who do not receive mother-milk. In this way the immune response development with respect to T-cell skewing and epithelial barrier function will be supported. As a result the risk of developing allergies or gastrointestinal disorders in a later stadium of life will be lowered

BSc projects:

- Investigate by analysis of available data (provided / literature) the fate of NDCs during *in vitro* batch fermentations using infant faecal inoculum

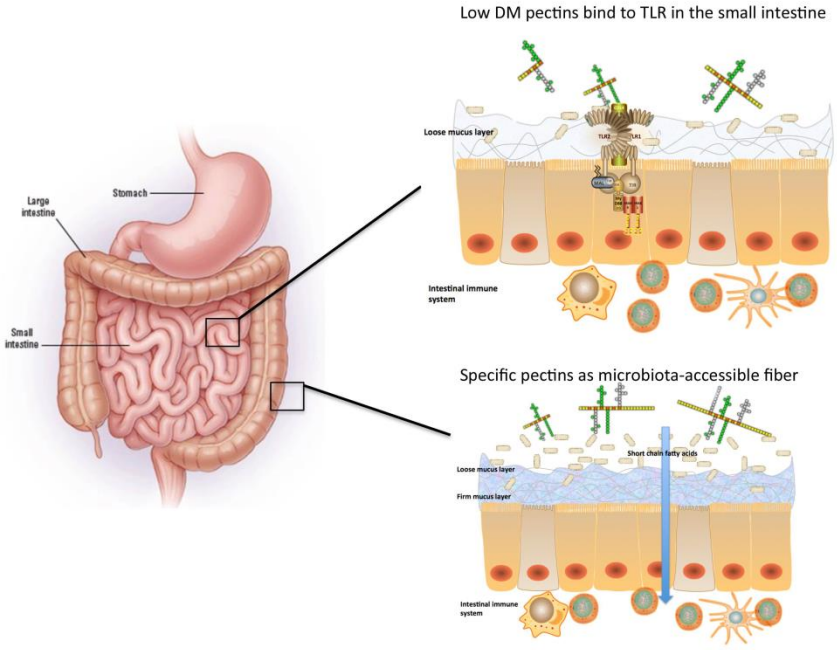
MSc projects:

- Characterization and quantification of NDCs by MALDI-TOF-MS, PGC-MS etc. Additional fractionation using AEC, SEC or preparative PGC-MS will be performed to unravel the different structures present in the highly complex mixtures.
- Following the fate of selected (fractions of) NDCs during in vitro batch fermentation using infant faecal inoculum by PGC-MS and SCFAs analysis using HPLC and GC. Unknown glycosidic NDC products will be identified by PGC-MS.

Supervisors:  Madelon Logtenberg,  Henk Schols

Topic 2.9 Carbs can make the difference: how pectins fuel immunity

A disbalance in microbiota communities in the intestine is implicated in a large number of Western diseases. This correlates with low intake of dietary fibre in Western diets. Pectin is a dietary fibre that might be essential for prevention of Western diseases. Recently, evidence has been found that beneficial effects of pectin are highly dependent on its chemical structure and the effects go beyond prebiotic effects. Earlier research has been demonstrated that low methyl esterified pectins have direct, microbiota-independent effects due to their direct interaction with TLRs in the small intestine. High methyl esterified pectins mainly impact the microbiota in the colon to enhance formation of pectic oligosaccharides and SCFA.



The aim of our research is to fully characterize bioactive pectins and their intermediate degradation products upon fermentation by enzymatic fingerprinting techniques.

Pectin molecules from various sources will be applied and enzymatically tailored with a combined beneficial effect. The already available data on pectin health effects will serve to produce a mixture of pectins with a desired molecular weight and methyl esterification pattern, which will be tested (in collaboration with the University Medical Center Groningen) in mice with a temporary disrupted small-intestinal barrier-function and mice with a disrupted colonic-integrity. Results obtained will be used to further tailor pectin structure to yield optimal bioactivity.

This study will contribute to the understanding of the structure-function relationship of pectins.

Possible BSc/MSc projects:
(For BSc: by analysis of available data (provided / literature). For MSc: including laboratory work)

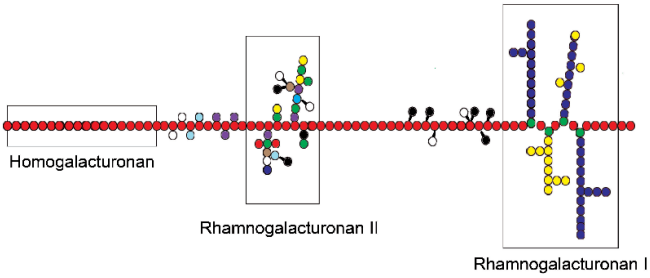
- Enzymatic tailoring bioactive pectic polymers.
- Chemical characterization of tailored pectins using enzymatic fingerprinting.
- Identifying and quantifying intermediate fermentation products from mice digesta & faeces of pectin fed mice.

Supervisors:  *Eva Jermendi,*  *Henk Schols*

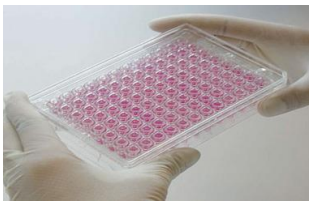
Topic 2.10 Structure-function relationship of immune active pectins

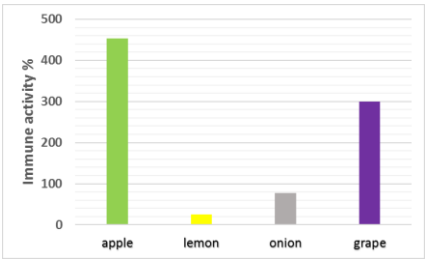
Pectins are polysaccharides present in cell walls of many different plants like apple, lemon or onion. Pectins are indigestible in the human upper intestinal tract and end up in the large intestine where they influence our health for example by stimulating our immune system. This immune effect is dependent on the detailed chemical structure of pectin and still needs to be unravelled in order to make health beneficial nutritional recommendations.
Typically, characterisation methods such as HPSEC, HPAEC, sugar composition analysis and determination of the degree of acetylation and methylation are used. Then specific enzymes are used to modify the chemical structure of pectin. Finally, immune assays of the modified pectins are performed by an external company and the chemical structures can be related to the measured immune activity.

Characterisation:



Immune assay:





Structure-function relationship?

BSc projects:

- Investigate by analysis of available data (provided / literature) the structure-function relationship of pectins from different sources and/or different chemical characteristics

Possible MSc projects:

In this project, you will study how the chemical structure of pectins are related to *in vitro* immune stimulation.

- Chemical characterization of pectins (molecular weight distribution, sugar composition, degree of acetylation and methylation).
- Enzymatic and chemical modification of pectin
- Investigation of the relation of chemical characteristics to immune stimulation (immune assays are performed by an external company)

Supervisors: Christiane Rösch *Henk Schols*

Topic 2.11 The effect of dairy protein composition on protein digestion kinetics

Proteins are digested in the gastrointestinal tract by proteases, ultimately leading to tri- and di-peptides and free amino acids (AA) that can be absorbed by the intestinal epithelium. The epithelium releases free AA into the blood stream. The kinetics of protein digestion and absorption appear to influence the fate of the AA systemically, i.e. incorporation in protein or oxidation and secretion as urea.

Protein digestion and absorption kinetics appear to depend on the protein composition. For example it has been shown in healthy volunteers that the postprandial AA plasma peak after whey protein ingestion is higher than after casein ingestion. This is usually attributed to the differences in gastric emptying between the proteins due to their distinct physicochemical behavior in the stomach. However, we have shown previously that in an *in vitro* digestion model enzymatic protein hydrolysis kinetics are also different between whey and casein proteins. This may contribute to the overall protein digestion and absorption kinetics as reflected in postprandial AA peak as well.

Enzymatic protein hydrolysis depends on the primary structure of the proteins. Whey and casein milk fractions are mixtures of proteins with unique primary structures. It is of interest to further the understanding of the effect of protein composition of protein mixtures on the overall enzymatic protein hydrolysis kinetics.

Possible MSc projects:

- *In vitro* digestion experiments will be perfomed to simulate gastrointestinal conditions. Protein hydrolysis kinetics of dairy protein (mixtures) will be monitored by SDS-PAGE, OPA, HP-SEC and UPLC.

Experiments will be performed at the life-science lab at Nutricia Research on Utrecht Science Park.

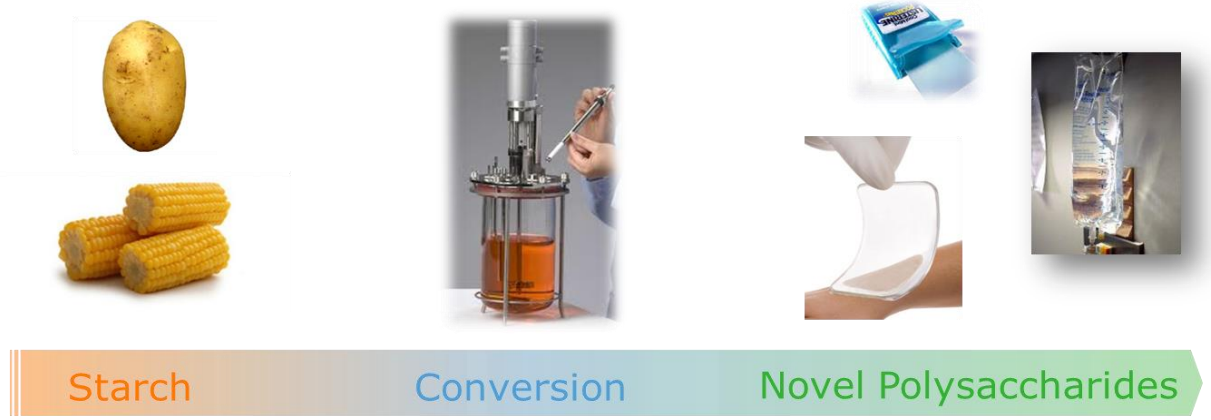
The life-science lab is a ML-II laboratory, therefore vaccination (Hep A+B, DTP) is mandatory. Not having valid vaccinations means they need to be arranged, via Nutricia Research, at the latest 6 wks before start.

Supervisors: Evan Abrahamse (evan.abrahamse@danone.com), Peter Wierenga

Part 3 Part 3: New and/or improved ingredients for food

Topic 3.1 IsoMalto/Malto-Polysaccharides (IMMPs): novel polysaccharides from starch

Starch is a natural polysaccharide that makes up a large part of the human diet. The compound itself is biodegradable and used in a wide variety of applications. Due to its relatively low price and its high availability starch is an attractive substrate for (enzymatic) modification.
The discovery of the GtfB enzyme (4,6-α-glucanotransferase) opens up a new way to modify starch. This enzyme is able to alter the intrinsic properties of starch by cleaving α-1,4 glycosidic linkages and introducing α-1,6 glycosidic linkages into the polysaccharide.
The products of this enzymatic modification are named IsoMalto/Malto-Polysaccharides (IMMPs), due to the nature of their linkage composition. IMMPs can be considered as a new generation of polysaccharides with added functionality for products in and outside the food industry, such as: food fibres, nutraceuticals, texturizers, replacement of synthetic polymers, biomedical materials and many other possible applications.



The aim of this project is to make the carbohydrates, to map the properties and to design optimal products for further applications.

Possible MSc projects:

- In depth chemical analysis of novel polysaccharides, including; molecular weight, ratio of α-1,4: α-1,6 linkages and directed (enzymatic) modification.
- Physical analysis of novel polysaccharides, including; viscosity, elasticity, gel strength, glass transition point and directed (enzymatic) modification.

Supervisors: Piet van der Zaal; Henk Schols

Topic 3.2 Monitoring the efficacy of non-starch polysaccharide (NSP) degrading enzymes to produce prebiotics in animal feed

Chicken feed includes cereals such as wheat, corn and barley, while soy and rapeseed can be added as a protein source. Besides starch, the cereals contain non-starch polysaccharides (NSP) such as cellulose and arabinoxylan. Soy and rapeseed also contain NSP, mainly in the form of pectin, cellulose and xyloglucan. The presence of NSP, especially soluble cereal arabinoxylan, increases the viscosity of the fluid in the gastrointestinal (GI) tract, resulting in reduced nutrient absorption. Supplementation of chicken feed by xylanases has been shown to reduce the viscosity in the GI tract, thereby improving chicken performance.

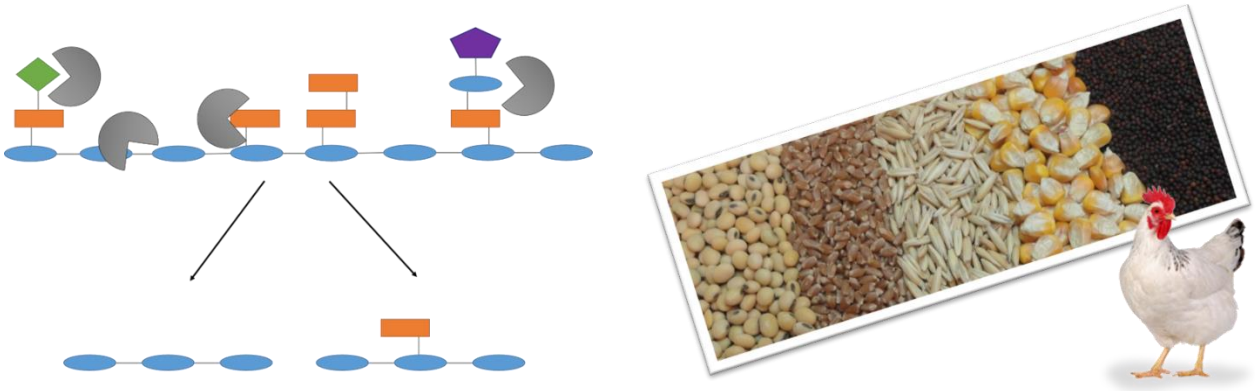


Figure 3. Schematic representation of the degradation of NSP by various enzymes, and the formation of the respective oligosaccharides (prebiotics)

However, the beneficial effect of the NSP degrading enzymes does not stop there; The degradation of arabinoxylan and pectin by endo-xylanases and pectolytic enzymes respectively, leads to the formation of oligosaccharides that are reported to exhibit prebiotic activity and immunomodulatory properties. Therefore, it is important to investigate the effect of different NSP-degrading enzymes on various feedstuff substrates. Also, we will look into the oligosaccharide profile produced by the aforementioned enzymes. Finally, the link between the presence of such oligosaccharides with the zoo-technical performance of chickens will be sought.

BSc/MSc projects:

- *Investigate for several situations the production of oligosaccharides by various NSP-degrading enzymes during digestion*

Supervisors: Dimitris Kouzounis Henk Schols

Part 4 Part 4: Educational development

Topic 4.1 Design and/or evaluation of a virtual experiment environment

The Laboratory of Food Chemistry is developing virtual experiment environments (VEE's). A VEE is a simulation that covers the complete process of performing research. In our food chemistry education, a VEE should motivate and enable students to prepare for laboratory education in an effective and efficient way.

In this project, you will combine three different fields: 1) food chemistry, 2) didactics and 3) information and communication technology (ICT). The goal is to design and/or evaluate a VEE. You will learn about educational theories, about using computer technologies and about a certain subject within food chemistry.

Possible projects (BSc and MSc):

- Designing and/or evaluating a virtual experiment environment for a course in the field of food chemistry. Depending on the interest of the student and the needs of the teachers we can describe the exact topic for the thesis project together.

Supervisors:  *Sjors Verstege,*  *Julia Diederen*

Topic 4.2 Developing new learning activities or improving existing learning activities

The digital learning environment of the laboratory of food chemistry, is continuously in development to improve education. Knowledge clips, screen recordings and digital cases are being used to facilitate the learning and understanding for many different topics. Where the knowledge clips and screen recordings are used to present the learning material in a conceivable way, digital cases are used to elucidate on the knowledge obtained.

The builder of these cases is responsible for the questions, hints and relevant feedback to the students. For this, you will gain in debt understanding of the topic and combine your field related knowledge with didactics and information and communication technology (ICT) in order to facilitate the learning process of students. Next to that you need to implement your creativity to create cases suitable and interesting to teach students.

Bachelor projects (not for Master thesis projects):

- Designing digital learning material containing enzymatic hydrolysis and chromatographic techniques required for structure elucidation of carbohydrates by enzymatic fingerprinting.
- Designing learning material for activating learning activities for a course in the field of food chemistry. Depending on the interest of the student and the needs of the teachers we can describe the topic for the thesis project together.

Supervisors:  *Bake de Rink,*  *Julia Diederen*