Research theme 4 - Phytochemicals in the food supply chains

Introduction
A large variety of **phytochemicals** (phyto = "plant" in Greek) are present in virtually all fruits, vegetables, legumes, and grains and are therefore easy for people to include them in their diet. Some phytochemicals are responsible for the typical color or sensorial properties in fruits and vegetables. Moreover, they are intensively studied for their protective or disease preventive properties. Major groups of phytochemicals are polyphenols, carotenoids, and glucosinolates. Many epidemiological studies have shown significant associations of diets rich in fruit and vegetables and reduced risk of certain cancers as well as several other chronic diseases like cardiovascular diseases and diabetes type 2 and obesity. To some extent these associations are ascribed to health promoting properties of various phytochemical actions like antioxidant, hormonal, enzyme stimulation, and antibacterial.

The concentration and composition of the phytochemicals in different plants, but also within a plant (e.g. in the seeds, roots or leaves), can vary greatly and also changes during plant development. Furthermore, various factors in the supply chain of vegetables including breeding, cultivation, storage and processing affect the intake and bioavailability of phytochemicals.

Glucosinolates
A specific group of phytochemicals called **glucosinolates** occur in representatives of the *Brassicaceae* family and are of particular importance in vegetables such as cabbage, Brussels sprouts, broccoli, cauliflower. Glucosinolates are claimed to be the active components responsible for many of the physiological effects proposed for Brassica vegetables in different types of studies, including in vitro, animal, human and epidemiological studies. Processing is one of the major factors affecting changes of glucosinolate content along the production and supply chain. Also domestic cooking methods influence the glucosinolate levels in various ways. Processing changes the glucosinolate content through several mechanisms. Each processing method involves specific conditions, which lead to various degrees of impact of the different mechanisms on the glucosinolate content. Using the underlying mechanisms that are critical for a processing or cooking method can be a valuable approach to understand the changes in glucosinolate profiles.

Overall objective
- To study mechanisms underlying changes in phytochemical content during industrial processing and domestic cooking practices
- To study the potential for increasing intake of health promoting phytochemicals, such as glucosinolates, by optimizing food processing to minimize losses.
Project 4.1 Investigating the effect of food matrix on glucosinolates thermal degradation

Target: MFT

Supervisors
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Glucosinolates (GSLs) are secondary plant metabolites that occur in members of the order Brassicales including Brassica crops, such as cabbage, rape, or broccoli. The consumption of Brassica vegetables is thought to be beneficial for human health because of breakdown products of the GSLs, namely isothiocyanates (ITCs). The latter being released enzymatically from GSLs by endogenous myrosinase after tissue disruption. During heat treatments (sterilisation, pasteurization, cooking) GSLs are degraded yielding a large array of breakdown products including nitriles, epithionitriles, isothiocyanates and the like. It has been shown that the chemical environment GSLs are in affect the rate of GSL thermal degradation which means that the same GSL will be degraded at different rates when occurring in different vegetal matrices. In addition, the relative amount of ITC and nitriles also may change according to the chemical environment.

Aim
In the present study we will investigate the effect of the vegetal matrix on thermal degradation of several GSLs.

Proposed approach
In a set of experiments, different vegetables (not containing GSLs) are selected, freeze dried and reconstituted with water and GSLs and subjected to heat treatments for different times. In a second set of experiments GSLs are dissolved in aqueous/alcoholic extracts from different vegetables and subjected to heat treatment. After heat treatments GSLs and ITCs are quantified in the samples and the relative effect of different vegetal matrix compared to the broccoli matrix.

Some references:
Project 4.2 Investigating the effect of food matrix on the conversion of glucosinolates to isothiocyanates

Target: BLT

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ITCs are the product of the degradation of glucosinolates catalyzed by myrosinase upon chewing and in general upon disintegration of the vegetal tissues. ITCs are supposed to be anti-carcinogenic. For that reason the amount of ITCs in the vegetables at the moment of swallowing has a great importance. The conversion of GLS to ITC depends on the chemical environment myrosinase and GLS are in. Supposedly different chemical environment would differently affect myrosinase catalytic activity and the final content in ITCs. It is long known for instance that vitamin C affects myrosinase activity. Analogously it is known that a set of proteins exists in planta which can direct the enzymatic conversion of GLS towards specific breakdown products (nitriles, epithionitriles) rather than ITCs.

Aim
In the present study we will investigate the effect of vegetal matrix in general and vitamin C in particular on the extent of the conversion of GLS to ITCs catalysed by the enzyme myrosinase.

Proposed approach
Different vegetables from cruciferous and brassicaceae are selected and their GLS content and MYR activity is assessed in different part of the plant. After grinding of the vegetable matrix the amount of ITCs produced and the amount of residual GLS are measured. The matrix effect is established by calculating the conversion rate, i.e. the % of GLS hydrolysed (or ITCs produced) per unit of myrosinase activity. In a different set of experiments selected (part of) vegetable will be grounded after addition of variable amount of vitamin C and the conversion rate will be calculated.

Some references:
Project 4.3 Effect of meal composition on the formation and bioaccessibility of isothiocyanates from broccoli

Target: BLT MFT

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Brassica vegetables are rich in glucosinolates (GLs), phytochemicals that can be hydrolysed by myrosinase (MYR), an endogenous plant enzyme. During mastication, plant cells are damaged and MYR catalyses the GL hydrolysis forming breakdown products including isothiocyanates (ITC), with anticarcinogenic properties.

A few small studies show the effect of different meal composition on ITC bioaccessibility. For instance, proteins may interact with ITC during digestion depending on the amino acid composition and the ITC type, reducing the ITC bioaccessibility. The in vitro digestion can be a useful tool to investigate the ITC formation and availability when eating broccoli along with other food components.

Objectives
The aims of to investigate the effect of broccoli meal composition (like proteins, fibre, fat) on ITC formation and bioaccessibility by means of in vitro digestion.

The student will work on the in vitro digestion to digest broccoli along with different meal components. GLs (HPLC), and ITC (LC/MS) at different stage of the in vitro digestion will be monitored.
Withanolides are a group of naturally occurring steroidal lactones which are mainly produced by the solonaceae family. Their name comes from the genera withania, that initially was considered to be the unique source. There is evidence, however, that the genus physalis also contains withanolides. There is high interest in these biocompounds since there is studies about their pharmacological activities, such as antitumor, anti-inflammatory, hepatoprotective, immunomodulatory activities, among others.

Studies have been done on the whole plant of physalis peruviana L. including fruit, suggesting that cape gooseberry also contains of withanolides, but the withanolide content of the fruits is still unknown.

A study to assess the contents of withanolides in cape gooseberry fruit and its processed product is needed to find out if this fruit and products have the special health promoting properties related to withanolides.

**Aim**

Set up the analytical method to identify and quantify withanolide. Apply this method to obtain the withanolide profile in cape gooseberry fruits and the effect of processing on this profile.
Project 4.5 Effect of heat treatment on In vitro bioaccessibility of β-carotene/vitamin C in cape goose berry

Target: BLT MFT

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Previous studies of vitamin C and β-carotene on gooseberry have given evidence on important contents of this health-promoting compounds in cape gooseberry. β-carotene has shown an increase during heat treatment that could be related to the degradation of binds of carotenes with other molecules. This bind degradation can lead to increase bio-availability of vitamin C and β-carotene.

Aim
Evaluate In vitro bioavailability β-carotene y/o vitamin C degradation during thermal processing.
As a healthy product but highly perishable commodity, diverse technology has been applied to extend shelf life of fruit. From the various existing processed fruit products, dried/dehydrated fruits are getting substantial attention, and recently the availability of those products in the market has increased considerably. Dried fruit is higher in energy per serving than fresh, canned, frozen fruit and most fruit juices. Their high content of dietary fibre contribute to more than 7.5% of the US daily value per serving (40 g). Most of them contain vitamins (e.g. vitamin A and C) and a wide range of phytochemicals.

The use of a combination of osmotic dehydration and heated drying techniques resulted in higher quality shelf-stable products to many type of dried fruits, compared to the application of only one of them. Among numerous attempts to improve the efficiency of osmotic dehydration process, the use of polysaccharide based-edible coating and food additives especially texture modifying agents (i.e. cell wall-modifying enzyme, Calcium (Ca) salts, phenolic compounds) shown good results.

Some of the research topics which involve experimental work in the laboratory might be, as followed:

1. Study on the effect of processing condition and maturity stages to the quality of dried fruit and its products on the physico-mechanical and phytochemical properties
2. Study on the effect of several process variables (incl. type and concentration of polysaccharides-based edible coating and texture modifying agents) to the quality of dried fruit and its products on the physico-mechanical and phytochemical properties.
3. Study on the effect of ternary compounds (sugars, several types of Ca-salts and weak acids) on the quality of osmotically dehydrated and/or dried fruit, especially toward the flavour release and colour degradation. This research might involve sensory evaluation.

Response Surface Methodology (RSM) will be applied to optimize important factors to have quality osmotically dehydrated and/or dried fruit according to their destined products.
4.7 Effect of Pulsed Electric Fields on tomato pulp

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Pulsed electric field (PEF) treatment is an innovative and promising technology for processing foodstuffs and consists in the delivery of short pulses in the range of μs to ms at different electric field intensities to a sample located between two electrodes. PEF is an alternative technology to thermal pasteurization due to its ability to inactivate bacteria, yeasts and molds (Saulis, 2010). Moreover, PEF has shown to slightly affect the activity of food-related enzymes (Clark, 2006). The literature reports many studies of the impact of PEF on biological activities and food quality. However, the comparison among the studies is very difficult due to large differences in experimental set up and especially processing parameters.

Aim
The aim of the present study is to investigate the effect of PEF on tomato quality parameters, such as colour, enzymatic activity, vitamin C, β-carotene and/or all-trans lycopene concentrations, as well as bioactives in vitro bioaccessibility.
In order to achieve 5 log reductions of the microbial load, PEF treatments will be carried out at 188.4 kJ/kg (Aganovic et al., 2014). Results will be compared to those obtained by applying a thermal treatment providing an equivalent pasteurization effect.

Experimental plan
Tomato juice will be subjected to PEF treatments under varying operating conditions. In particular, electric field strength, number of pulses, and solid soluble concentration will be varied according to a three variable face centred central composite design and the effects of these variables on vitamin C and carotenoids concentrations, and colour will be evaluated. The evaluation of the effect of PEF on the chosen dependent variables will be used to achieve the optimal conditions to obtain tomato juice with high quality in terms of vitamin C retention, colour and carotenoids concentration.
Furthermore, the PEF treatments allowing the best quality parameters to be achieved will be compared to a thermal pasteurization treatment at time zero and during storage at 20 °C up to 100 days. In particular, pectin methyl esterase (PME) activity, colour, vitamin C, β-carotene and/or all-trans lycopene concentrations and in vitro bioaccessibility will be evaluated.

Selected references
Tropical fruit has been widely recognized for their sensorial quality and their health benefits, being an important source of carbohydrate, vitamins, minerals, fibers and a large number of bioactive compounds that contribute to human health. On the other hand, in Europe fresh tropical fruit supply is limited due to transportation and the quantity. Furthermore, Indonesia as a large producer of tropical fruits such as pineapple, mango, avocado and papaya has a minimum contribution to the European market. Due to a limited shelf life and the necessity of air transport, fresh fruit needs to be processed to prolong the shelf life and increase its value. Attention to various processes that enables to maintain its health and nutritional benefit has been increased. Vacuum frying is an emerging technology of innovative processing in recent years for fruit and vegetable products. Vacuum frying may process fresh fruit into chips that have low moisture and fat content, while preserving health benefit.

Effects of some processing variables such as frying temperature, vacuum level, frying time and pre-treatments to the fruit chips has been investigated. Fruit properties, structural, composition, and bioactive compound content will be changed during ripening. Yamsaengsung, Ariyapuchai have studied the effect of ripening on physical and textural changes of the product. Starches that converted to sucrose and maltose during banana ripening affect the product expansion and diameter shrinkage. Though, the effect to the vacuum fried fruits quality attributes such as color, texture, structures and bioactive compounds, during vacuum frying has not been studied.

Some of the research topics which involve experimental work in the laboratory might be, as followed:

4. Study on the effect of ripening stages to the quality of vacuum fried fruits on the fried products on the as color, texture, structures and bioactive compounds properties
5. Study of kinetic of the bioactive compound during vacuum frying process.

Since fruits used for this thesis will be banana, avocado, mango, pineapple, and watermelon. the project will need five students.
4.9 Functionality of carrot fibres and influence of extraction technology

Supervisor
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Valorisation of waste streams is gaining more attention the last years. Under the slogan “food should remain food” we investigate the possibilities to (re)use or convert vegetable waste streams originating from farmers, trade and industrial processing. Many vegetables contain a high amount of fibres which could be used as sugar/fat replacers in food or as structuring components. In this project we investigate the properties of carrot fibres in relation to application in food products. The influence of the extraction technology to obtain the fibres is investigated as well (drying temperatures, extraction temperature and time). The properties are compared to properties of current fibre sources for food (such as wheat and citrus). The response of the properties on process variations are used to create to model to optimise process settings.