

# Sustainable Protein Technology

Report 1786

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An evaluation on the STW Protein programme and an outlook for the future



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# Abstract

In 2013 a new STW research programme was started on sustainable protein recovery. This STW Protein Programme consisted of five sustainable protein technology projects, which aimed at developing innovative methods to extract proteins from plant leaves, microalgae and insects to meet the increasing demand for food proteins for humans and livestock.

The aim of the additional STW-project '*Meer en Beter Eiwit*' was to summarize and evaluate the main results and conclusions of these five projects. Besides, some more recent additional insight on protein extraction was supplemented. Project partners including WUR, knowledge institutes and industry were interviewed to obtain their opinion on the project performed and future research needs. This has led to a vision document that gives direction to future research in the field of protein and technology.

The approach of this project was to study the topic from start (biomass) via technology to finish (product). It was further put into a larger perspective, looking at the entire chain. When relevant, additional aspects such as soil quality and global protein demands were included.

## **Biomass choice**

When choosing a particular protein-recovery technology, biomass selection is the first requirement. Much research is being done on new biomass. However, the use of existing raw materials and residual flows should not be neglected. By building on existing processes and chains, fast(er) implementation is possible. Traditional crops like grain are relatively dry, and the proteins are present in protein bodies. Therefore, they are more easily extracted and give high yields and purities. New, green crops still require a lot of development.

## **Protein streams in the world**

Protein-rich sources, like soybeans, rapeseed, maize and wheat are being consumed by both humans and animals. The crop with the largest production volume in Europe is wheat, followed by potato, maize and barley. Together these four crops cover about 85% of the production of protein crops. Worldwide, maize is the largest crop. By far, the largest amount of proteins is being used in feed (>75%), followed by food consumption. Only a limited amount of proteins is isolated for specific use, for example as

emulsifiers in different food formulations. An even smaller amount is used for application in chemicals or materials.

## **Protein purity and functionality**

Much research from the past focused on obtaining pure protein, e.g. RuBisCo from green leaves or protein from potato juice. Such processes can be economically feasible if the protein produced has a specific functionality, which allows for use as a high-quality food ingredient. However, high purity is not always required to obtain a certain functionality. In such cases the use of less refined, functional fractions is an interesting alternative.

## **Mild separation and fractionation**

When purified components are replaced by functional fractions, less intensive separation conditions can be used. Dry separation of proteins yielding a concentrate could be an alternative to wet separation yielding an isolate. Energy consumption is less, and a more native protein can be obtained. Fractionation can also lead to more complete use of biomass, generating little to no side streams.

## **Chain approach for economy and sustainability**

Next, it is also important to include the possibility of complete utilization of the raw material and closing cycles. These latter aspects can make or break economic feasibility and sustainability in a process. Efficient and effective use of protein and nitrogen, while maintaining biodiversity, is the most important development point for sustaining life on this earth. Modern agriculture should further improve nitrogen and feed use efficiencies to increase sustainability.

## **Program evaluation**

Next to conclusions on the content of the five projects, the evaluation also provides conclusions on the set-up of the STW/EZ programme on sustainable protein. Both academic and industrial participants acknowledged the added value of the link between fundamental research by a PhD and applied research by research institutes that was made in the project set-up.. They also partly attributed the project successes to the multidisciplinary approach in the projects. The possibility within the projects to look at all aspects, and the ability to think anew on existing processes and develop new concepts of biorefinery greatly added to current scientific knowledge on protein extraction.

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# 1 Introduction

The world population continues to grow and along with it the demand of proteins for human consumption. Considering that also the number of people eating meat increases, this results in an even higher demand for protein, since for the production of 1 kg of beef, about 7 kg of soy are needed. As a consequence, we need a durable protein supply for humans and animals and we need to be able to provide for protein shortages. In 2013 a new STW (Currently NWO Domain Applied and Engineering Sciences) research programme started on sustainable protein recovery.

The programme challenged scientists and entrepreneurs to force a breakthrough in the field of sustainable protein production with new knowledge, technological inventions and practical applications. This should enable delivery of protein for food (or feed) or food ingredients in an ecological and economical sustainable way. The upgraded protein should have a higher economic value than its starting material. Five projects were granted that concerned the development of innovative methods to obtain proteins from plant materials, micro-algae and insects to meet the growing need for food for humans and livestock.

## **The five original projects were (STW, 2012):**

- Progress - Proteins from green sources for use in both food and fish feed, Main applicant prof.dr.ir. Harry Gruppen
- In2Food – Production and valorisation of high quality proteins from insects, Main applicant prof. dr.ir. Tiny van Boekel
- GreenProtein – Isolation of hydrophobic proteins from green plant materials, Main applicant prof. dr.ir. Remko Boom
- LEAP – Increasing the utilization of low value leaves by use of new protein extraction methods, Main applicant prof.dr.ir. Johan Sanders
- ALGAEPRO4YOU – Extraction and fractionation of functional proteins from microalgae for potential application in the feed of food industry, Main applicant dr.ir. Marian Vermuë

Next to a team of researchers, also a PhD was appointed for each topic. Most of these projects are now (almost) finished. From October 1 2016 until September 2017, a sixth project started with a team of WUR researchers.

## **1.1 Finalizing project: More and Better Protein**

Finalizing the STW Protein Programme, the '*Meer and Beter Eiwit*' project aimed to give background knowledge on protein use and technologies for recovery, an overview of the research conducted within the programme, and an outlook for future research.

The background knowledge is provided at the start. In Chapter 2, we situate the global protein problem, by looking at the importance of the nitrogen cycle. This gets illustrated by new research on the current Dutch protein/nitrogen flows and efficiencies. After setting the scene, an overview is given of all biomass types that are important for protein production in Chapter 3. This is followed by Chapter 4 on protein technology. This chapter gives an overview on the background protein technologies relevant to the work performed in the previous five STW protein projects. It also provides important results and the conclusions drawn from it. Chapter 5 is then focussing on the product, in which we aim to provide insights in product prices and required functionality. Chapter 6 is the first chapter that starts to look forward to future research. For this we looked at the results from the projects and advice given to us by both researchers and industry. This led to the final conclusions and recommendations that can be found in Chapter 7 and that are based on all previous chapters.

In addition to this report the project also yielded two other scientific publications:

Lesschen, J.P. and Sanders, J.P.M. 2017. Options to



improve the protein use efficiency in the Dutch agriculture sector. Report Wageningen Environmental Research, Wageningen.

Tamayo Tenorio, A., Kyriakopoulou, K.E., Suarez-Garcia, E., van den Berg, C. and van der Goot, A.J. 2018. Understanding differences in protein fractionation from conventional crops, and herbaceous and aquatic biomass - Consequences for industrial use. *Trends in Food Science & Technology* 71: 235-245.

# 2 Protein and nitrogen flows and efficiencies

Protein shortage, or more general nitrogen (N) shortage, is not always recognized as one of the most important sustainability issues. The general public tends to focus more on greenhouse gas emissions (specifically CO<sub>2</sub>) and loss of biodiversity. In this chapter we aim to demonstrate the importance of global sustainable protein use, illustrate it with numbers from our Dutch agricultural system, and add suggestions for improvement. Technological solutions, like biorefinery for protein is one of them.

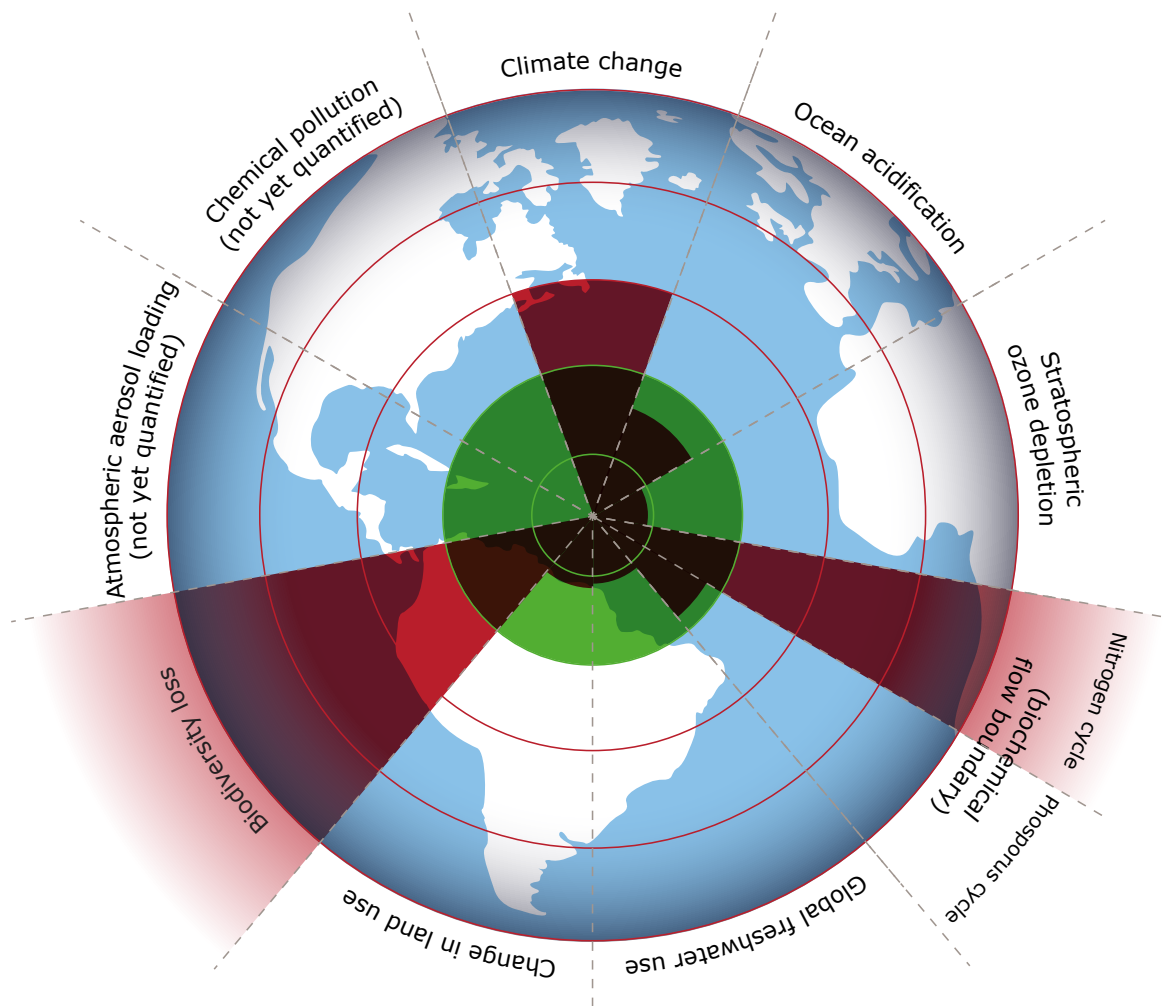


Figure 1: Beyond the boundary. The inner green shading represents the proposed safe operating space for nine planetary systems. The red wedges represent an estimate of the current position for each variable (Rockström et al. (2009)).



## 2.1 Safe operating space for humanity: Nitrogen

Rockström et al. (2009) describe a safe operating space for humanity. In their evaluation of nine planetary systems they conclude that the boundaries in three systems (rate of biodiversity loss, climate change and human interference with the nitrogen cycle), have already been exceeded (Figure 1). In this respect human disturbance of the nitrogen cycle is the second most interfering process after biodiversity loss.

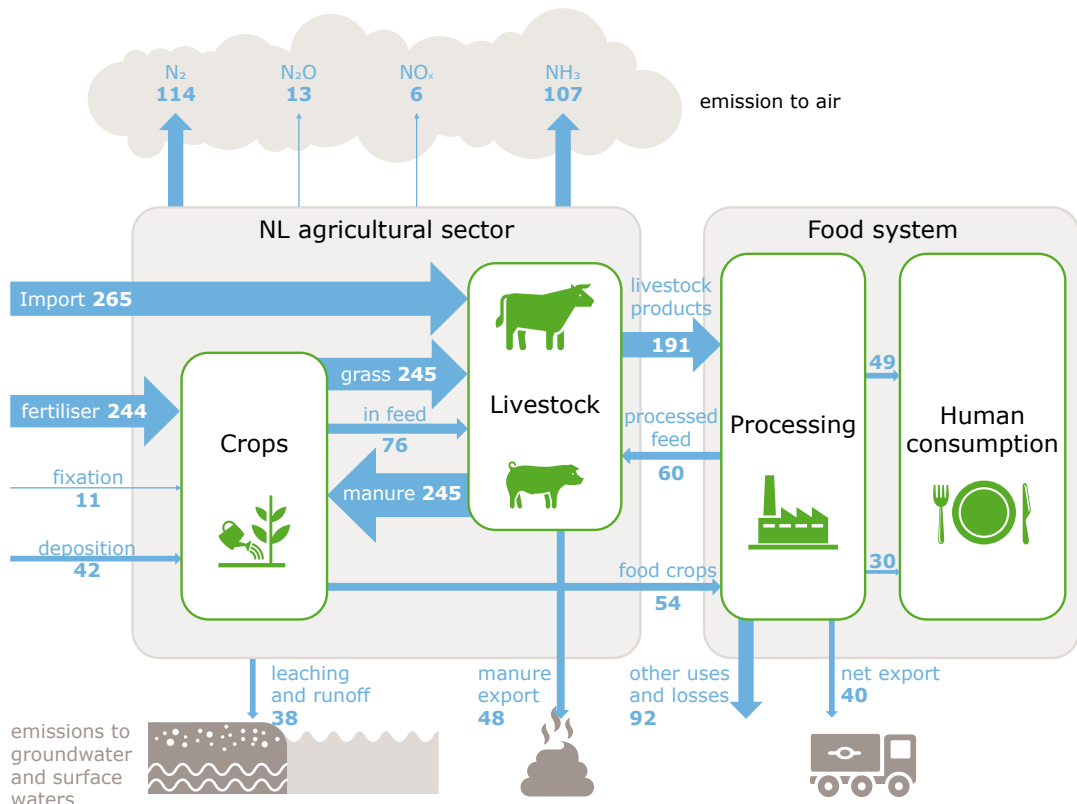
According to Rockström et al., modern agriculture is the main cause for the disturbance of the nitrogen cycle. Around 120 million tonnes of  $N_2$  from the atmosphere per year gets converted into reactive forms, which is more than the combined effects from all Earth's terrestrial processes. Much of this new reactive nitrogen ends up in the environment, polluting waterways and the coastal zone, accumulating in land systems and adding a number of gases (e.g. nitrous oxide) to the atmosphere. It slowly erodes the resilience of important Earth subsystems.

More sustainable use of nitrogen in agriculture is thus a necessity for the future. We conducted a short study on the current situation in the Netherlands and on how to improve nitrogen use efficiency. Below is a summary of this. The complete study will also be published in a separate publication (Lesschen and Sanders, 2017).

## 2.2 Options to improve protein efficiency in Dutch agriculture

With a growing world population and changing diets, the global demand for animal proteins for human consumption is expected to increase. However, the production of animal proteins has a significant impact on the environment, including expansion of agricultural land, greenhouse gas emissions, eutrophication of surface waters and nutrient imbalances. The nitrogen (N) recovery in animal farming is inherently lower than in crops, with only 10–50% of N in feed being retained in live weight and 5%–40% in the edible weight. Several options exist to improve this efficiency, either by reducing N losses, reducing external N inputs (such as mineral fertilizer), or improve the feed conversion.

Figure 2. Nitrogen flows (kton N) in the agricultural and food system in the Netherlands for the year 2014.



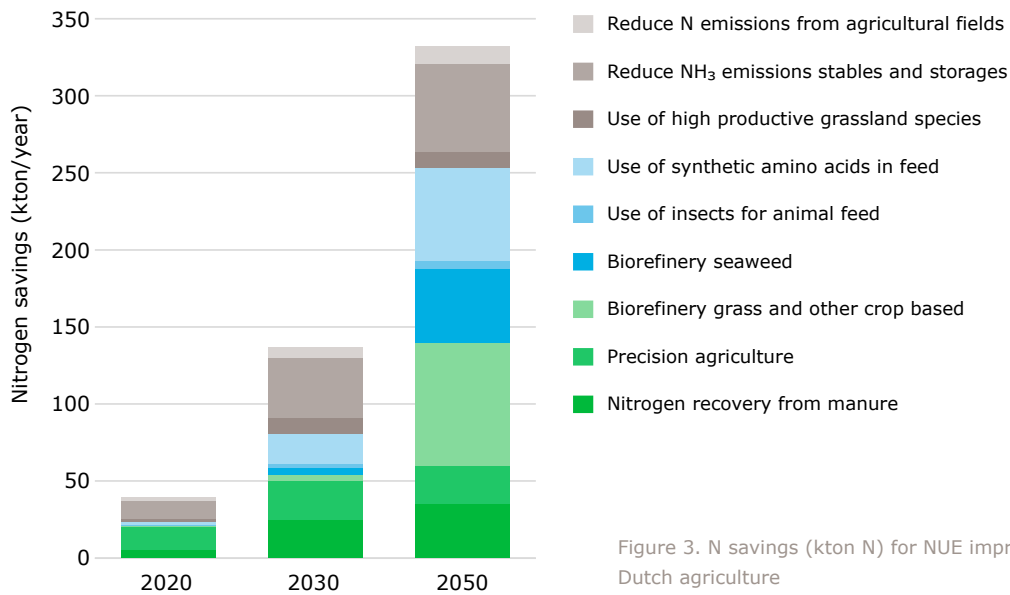
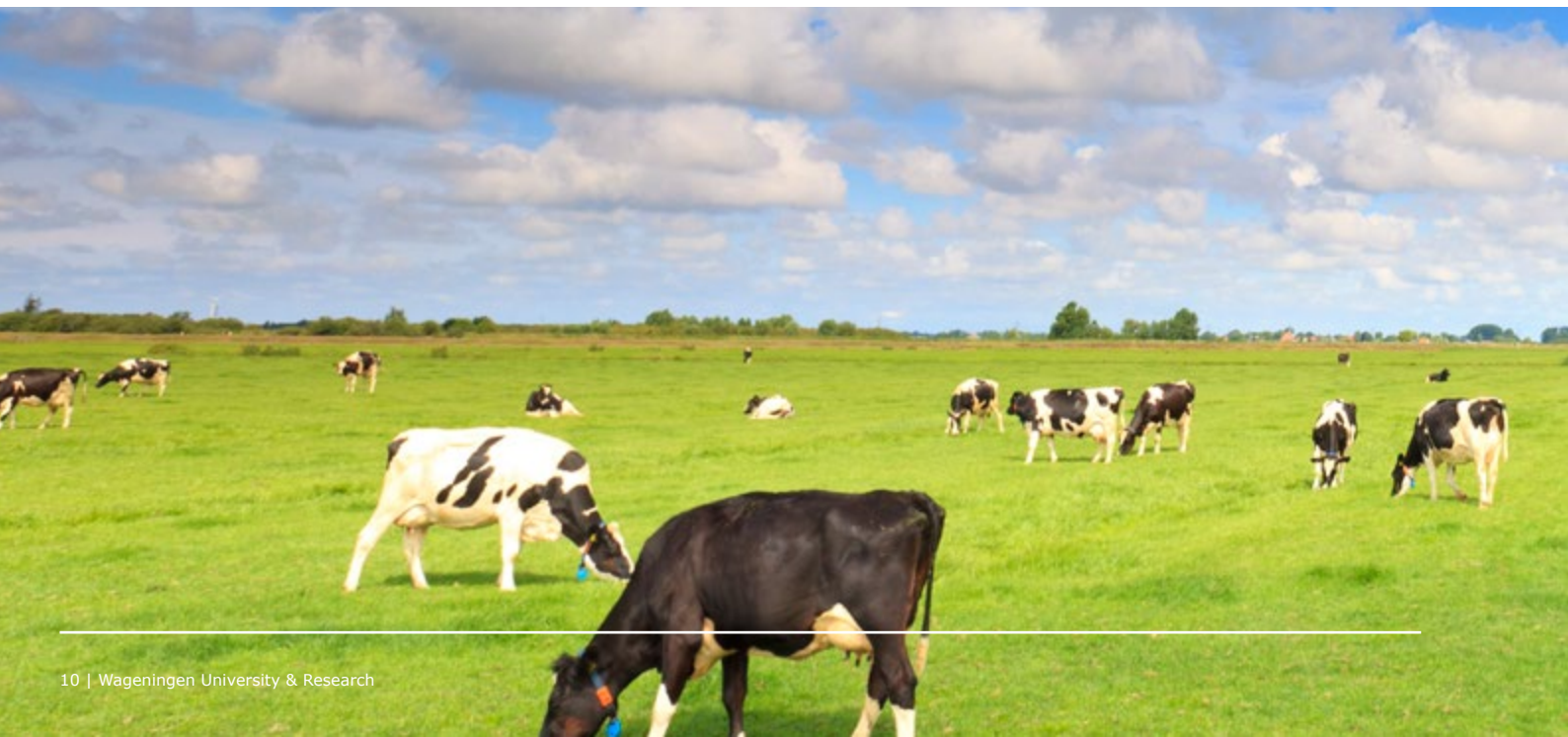


Figure 3. N savings (kton N) for NUE improvement options in Dutch agriculture

In the Netherlands nitrogen and feed use efficiencies have already improved over the last decades. The soil N surplus (N inputs minus crop N uptake) reduced from more than 600 kton N in 1990 to about 300 kton N in 2015. This reduction has mainly been achieved by more strict manure and fertilizer policies. Nevertheless, water and air quality still need to be improved further in several regions, and climate change mitigation is becoming more important. The aim of this study is to quantify the nitrogen flows and nitrogen use efficiency in Dutch agriculture and to assess options to improve this efficiency. This study

contributes to the overall STW protein programme by providing recommendations for further research on improving protein efficiency.

The analysis of nitrogen flows and efficiencies is based on the existing nitrogen flow scheme for the EU from Westhoek et al. (2014). For this study we focussed on the Netherlands and used the environmental impact assessment model MITERRA-NL to quantify the different nitrogen flows for the Dutch agricultural system. The starting point of this study is to maintain the current food consumption and production of proteins, but



reduce the external inputs to the system, i.e. mineral fertilizer use and import of proteins from outside the Netherlands. Different improvement options were identified and their impact was estimated for 2020, 2030 and 2050, where the last one represents the technical potential. The following improvement options were assessed, which were grouped into three main categories:

- 1 Options to reduce N mineral fertilizer use
  - a. use of mineral concentrates
  - b. Alternative use of poultry manure
  - c. New N fertilizer products from animal manure
  - d. Recycling of human waste as fertilizers
  - e. Precision agriculture
  - f. Use of N fixing crops
- 2 Options to reduce import of feed
  - a. Biorefinery (grass, crop residues, duckweed and azolla, seaweed and green manure)
  - b. Ban on maize as co-substrate for biogas
  - c. Use of insects for animal feed
  - d. Use of synthetic amino acids in feed
  - e. Higher yielding grass species
- 3 Options to reduce N emissions
  - a. Chemical or biological  $\text{NH}_3$  filters on stables
  - b. Acidification of manure storages
  - c. Stripping of ammonium during manure treatment
  - d. Catch crops
  - e. Nitrification inhibitors

The current nitrogen flows in Dutch agriculture show that import of feed and mineral fertilizer use are the two main external sources of nitrogen input and gaseous emissions to the environment the main losses (Figure 2). Current nitrogen use efficiency in the crop system is about 60%, whereas in the livestock systems only 30%. Our results show that the improvement options could reduce the external inputs of nitrogen by 40 kton in 2020, 135 kton in 2030, whereas the technical potential by 2050 is estimated at 330 kton N. On the short term (2020) reduction of N emissions and more efficient use of manure are the main improvement options, whereas on the longer term biorefinery for more efficient use of protein, new sources of local protein (e.g. seaweed) and synthetic amino acids in feed can contribute most to the further reduction in external N inputs (Figure 3).

These findings show that there is still ample space for improvement in nitrogen use efficiency in Dutch agriculture, which will reduce the nitrogen emissions to the environment. However, many of the improvement options are not yet cost-effective or still need to be further developed. This requires a holistic approach, with collaboration and multi-disciplinary research programmes to reduce cost and conflicting regulatory issues.



# 3 Protein containing biomass

With the growth in world population and its affluence, more protein is required for food. As people tend to eat more meat, more protein is also needed for feed. Alternatives on protein resources and direction on protein usage for specific needs are therefore required. Protein from edible plant parts can be directed to food while the remaining biomass can be used for feed or chemicals.

The use of by-products as protein resources can provide additional proteins, possibly after a bio-refining process. However, the remaining challenge in protein refinery is the optimal extraction of protein from biomass.

Protein-rich sources, like soybeans, rapeseed, maize and wheat are being consumed by humans and animals for the necessary amino acid uptake. In Europe, the largest crop is wheat, followed by potato, maize and barley. Together these four crops cover about 85% of the production of protein crops. Worldwide, maize is the largest crop.

Biomass resources rich in proteins (>10 wt%) are currently mainly used in the food and feed sectors, however, their use is often very inefficient. By the separation of protein fractions from these resources and by upgrading to specific food and feed ingredients, using the residues for valorisation to other biobased outlets, the biomass resources can be used more sustainably. Within the 'Circular Bio-Economy' sustainably produced biomass is used for the co-production of a portfolio of human food and animal feed ingredients, chemicals, materials, advanced biofuels for transport, power and heat using efficient and closed-loop biorefinery processes. Therefore, innovative biorefinery research programmes are inventing new ways to extract proteins from protein-rich sources. These initiatives have their focus on the extraction and isolation of proteins from different types of biomass, such as side streams from the oil production, from the bioethanol production, from

the starch industry, or from herbaceous and aquatic biomass. (adapted from Mulder et al., 2016)

## 3.1 Oil sees meals: established source of plant protein

After oil, protein is the second largest component that is present in oilseeds such as rapeseed, and sunflower; and with no exception in soybeans, which are generally considered as oilseeds. The oil in the oilseed is commonly collected by solvent extraction, usually with hexane. After oil extraction, hexane remains are removed by desolventization. This is done at high temperature, causing denaturation of protein. Following oil extraction, oilseed contains as much as 30–50% protein. With this high protein value, oilseed is one of the most important plant protein resources and is already used as such. In terms of production, soybeans are by far the most important legume. Soy protein products range from full fat soy flours (having a protein content of about 40%) to soy protein isolates (protein contents above 90%). Defatted soy flours, which are mainly used in feed but also in some food products, are the most widely produced flours. They are prepared by milling defatted soy flakes. Soy protein isolates are obtained by alkaline extraction from defatted soy flakes followed by precipitation of proteins at their iso-electric point. The precipitated proteins are, usually after neutralization, spray dried. Additional processing steps may be incorporated (e.g. jet cooking prior to spray drying) to improve functional properties of the proteins. Soy proteins are mainly used as a



functional protein ingredient in a large variety of food products.

Soy protein concentrates (65% protein) are obtained from defatted flakes by removing soluble carbohydrates. The functional properties of soy concentrates are tuned by processing steps like neutralization, steam injection, and mechanical shear through homogenization. Further processing is leading to higher purity fractions known as isolates. Similarly, the functionality of soy isolates can be optimized by enzymatic hydrolysis, reduction of disulphide bonds or heat denaturation. The target functional properties might require one or several modification steps. The final product is spray-dried into a flowable powder, and the drying conditions are critical to retain protein functionality (Thrane et al., 2017). Furthermore, soy flour, concentrates, and isolates are transformed into products with defined textures through extrusion processes, steam injection, jet cooking, shear technology or extruding into an acid-salt bath that coagulates protein into fibres (spinning process).

Nutritional wise the quality of protein is based on the number and types of amino acids it contains. Animal foods contain high-quality protein, and egg white has the highest quality protein of all. Unlike other plant or vegetable proteins, soy protein contains all of the essential amino acids necessary for good health. That makes soy protein a complete protein, similar to the protein found in animal foods. Soy protein ingredients can replace animal-based proteins (meat, dairy, and egg) to certain extent, while reducing product costs and improving nutritional profile, functionality and sustainability. In terms of functionality the partial replacement of animal protein, in meat patties for example, has to do with higher water retention provided by soy protein ingredients, limiting the percentage that can be incorporated into a formulation. To provide different functions for food products (e.g. emulsification, gelation, water binding, foaming, viscosity) the appropriate process is applied to modify the protein isolates. Food products containing soy protein ingredients include salad dressings, soups, nutrition bars, meat analogues, beverage powders, cheeses, non-dairy creamers, frozen desserts, whipped toppings, infant formulas, breads, breakfast cereals, pastas, and pet foods (Thrane et al., 2017).

### 3.2 Cereals: abundant and easily extracted protein source

Cereals have a much lower protein content than oilseeds, typically about 10–12%. This low value is the reason that cereals have been underutilized as a protein resource. However, with the abundant amount of cereal production for both food and biofuel production, vast amounts of cereal protein is and will be available. With 1500 million tonnes of cereal (rice, wheat, and oat) production in 2013, about 180 million tonnes protein is produced. This value is comparable to the 200 million tonnes oilseed protein, coming from 400 million tonnes oilseed (soybean, rapeseed, and sunflower) production (FAO website).

Industrially, cereals are being used for the production of starch. Aqueous treatment washes off cereal starch and leaves the insoluble protein; such as gluten in wheat. Cereal proteins are rich in sulphur containing amino acids. The presence of these amino acids results in formation of disulphide cross-linking that lowers protein solubility. The use of alkali can break these linkages and in addition ionize all neutral and acidic amino acids, thus increasing protein solubility.

Prolamins are the major storage proteins in most cereal seeds, and as such are an important source of dietary protein for man and livestock (Shewry et al., 2002). For food and feed applications by far the most commonly used is wheat (gliadin) while other prolamin containing cereal seeds include barley (hordein), rye (secalin), corn (zein), sorghum (kafirin). Prolamins of wheat are the major components of gluten, the properties of which determine the quality of wheat flour for various technological processes (Shewry et. al, 2002). Gluten proteins are an example of a substance whose favourable and usable characteristics are combined with properties that are unfavourable or even harmful to health. These proteins compose of monomeric gliadins and polymeric glutenins. They have a unique technological role in food applications with the most important in bread making. Elastomeric protein groups interact, forming viscoelastic gluten matrix having properties that directly affect the quality of bakery products (Kàczkowski, 1991). Foods made of wheat seed are common in human

diets, hence gluten proteins are nutrients of great importance. Unfortunately prolamin proteins, especially gliadins, may be strong food allergens . Their specific properties cause two types of undesirable food reactions to gluten. The first include IgE-mediated allergies, such as asthma, food allergy or anaphylaxis, which constitutes direct mortal danger (Bürk et al., 2001; Varjonen et al., 2000; Vissers et al., 2001). Secondly, these proteins may aggravate coeliac disease, a genetically-determined gluten-dependent disease in which proteins damage intestinal membranes and degenerate villi, causing serious disorders of the alimentary system (Wieser, 1996; Shewry, 2011; Shewry et al., 2002).

### 3.3 Pulses: traditional protein source with renewed interest

Grain legumes are cultivated for their seed. These pulses are used for direct human consumption and contain relative high amounts of protein, which makes them suitable as an alternative source of

protein. Within Europe firstly pea and secondly faba bean are the most cultivated grain legumes. They contain 20-25% protein.

Legumes go through several processes before they are ready to be used either as a plated item or as an ingredient in food preparations. These processes can include cleaning, drying, sorting, possibly dehulling, splitting, milling, and finally fractionating. The latter is done to obtain protein concentrates and isolates. Fractionation typically takes the form of a dry (air classification) or wet method (wet milling). The dry method can be more sustainable as it uses less water, yields a partially pure concentrate (50-70% protein) with protein in a native form. The traditional wet process to prepare plant protein ingredients starts with a dispersion in water to dissolve the protein and suspend the starch granules, with a subsequent precipitation of the protein. This yields a high purity protein isolate (80-90%) that is denatured. (Boye et al., 2010). The wet processing method for starch is also used to obtain high purity fractions from other sources



like potato. Combining industrial processing of potato with processing of pea or faba beans can lengthen the processing season, and solve some of the down time issues that starch industry has due to seasonality of their biomass source.

The obtained high protein fractions from these sources are considered fairly functional when compared with other protein sources, while the processing is considered economically viable. Protein fractions from legumes are being applied in food products for a great variety of applications. Moreover, projections predict the increase of protein-rich products using pulses as protein source (Mulder et al., 2016). Acceptable flavour often proves to be the biggest development challenge (Egbert and Borders, 2006) for food applications. From a consumer perspective, acceptable flavour though is not the same across different regional markets. For instance high protein soy products in Asia with strong soy characteristic flavour are considered superior. On the contrary, in Europe similar food products are considered better with milder or even masked soy flavour. Based on the above, food industry has addressed these challenges by a combination of different techniques: addition of fragrances and aromas, identifying aroma-protein interactions and masking of flavours. The combination of these techniques provides flexibility on the formulation of the finished product.

### 3.4 Novel protein sources

There is an increasing interest in new sources of protein-rich biomass that is caused by the demand for alternative protein sources for food and feed and the increasing interest in protein usage for bio-based applications. The biorefinery of most of the new protein sources, however, is still in the research phase. For food and feed applications, 'new' potential protein sources are several leaf proteins (grass, beet leaves), aquatic biomass (seaweed and micro algae), insects (mealworm) and single cell proteins (fungi, bacteria, yeast). These proteins could provide new and sustainable food ingredients for humans to meet the growing demand for protein.

#### 3.4.1 Leaves: abundant but difficult to extract

Green plants and leaves have a huge potential for production of proteins, due to their vast

availability. Important crops that can be used for the production of proteins are grass, beet leaves, alfalfa, and spinach leaves.

Protein products from leaves are usually obtained by pressing or shearing the fresh crops to remove the fibres, followed by recovery of the proteins from the liquid fraction by heat coagulation, centrifugation and drying. Because of the coagulation process that is performed at elevated temperature, the proteins lose most of their functional, but not their nutritional properties. Grass and lucerne juice has been fed to animals and it was shown that these leaf proteins could function as an alternative for e.g. fish protein or soybean meal.

Although the amount of easy-to-solubilize protein from leaves may be low when only water extraction is used, the addition of alkali can increase protein extraction yields. High yields can be obtained, when a combination of high temperature and alkali is applied.

The most abundant protein in leaves is an enzyme; Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) which is involved in the first major step of carbon fixation in plants. RuBisCO constitutes half of the water soluble proteins in leaves and its extraction and characterisation has been extensively studied. The solubility of RuBisCO depends on the extraction and drying conditions. RuBisCO from spinach leaves shows the lowest solubility between pH 3-5, and goes up to 100% at pH 7 (van de Velde et al., 2011). RuBisCO obtained after freeze-drying or mild spray-drying (80°C) has low water binding capacity, which seems to correlate with RuBisCO's high solubility; while drying at higher temperatures causes protein denaturation and subsequent increase of water binding by RuBisCO protein (De Jong & Nieuwland, 2011). Compared to soy protein, RuBisCO from alfalfa leaf has higher oil binding capacity, better emulsifying properties and strong gel formation at low protein concentrations (Knuckles & Kohler, 1982). Nevertheless, extraction conditions greatly define the final functionality.

#### 3.4.2 Microalgae: Source for alternative protein that is hard to obtain

Microalgae are easy to cultivate, fast growing, and having high energy per hectare leading to



considerations for use as energy resource. Protein concentration in algae can be as high as 40–60% w/w based on dry weight, depending on the strain used.

Historically, microalgae have been considered as a source of proteins for foods, but were not considered interesting because of the co-extracted colour from non-protein components. Interest in microalgae was renewed when they might be grown for fuel production due to their oil content, and their protein fraction was studied more intensively once again. Recently, an improved bead milling method has been developed to extract protein from *Tetraselmis* sp. with emphasis on discolouring the microalgae protein isolate to meet a broad range application of the final isolate. This developed method still requires optimization as only 21% protein was extracted into the liquid phase.

Generally speaking, algae are hard to disrupt and protein extraction efficiencies are not high. This is mostly due to their cell wall, which is of a different composition than plant cell walls. Only a few algae are without a cell wall (Sari, 2015).

#### 3.4.3 Insects: The animal alternative

Edible insects constitute an alternative protein source for human food and animal feed with advantages that include low greenhouse gas emissions and land use, high feed conversion efficiency, and their ability to transform low value biomass into high value protein products (van Huis, 2016). However, not all residual biomass is suitable. Growth on manure is e.g. less efficient than on food processing by-products such as distilled grains (Smetana et al., 2016). It is also less desired from a food safety perspective. Additionally, edible insects can acquire toxic substances or human pathogens from their feed, which necessitates a hygienic production and limiting the type of waste stream used.

In the European Union (EU), insects are not yet allowed for feedstock for pigs and poultry, but they are used as aquatic feed since 2013 (van Huis, 2016). In the end of 2015, the EU decided that insects have to go through Novel Food regulation for food applications, although national exceptions exist. People allergic to crustaceans or house dust mites might be allergic to some insects as well.

As a protein source, edible insects have comparable protein content as meat, and they contain more polyunsaturated fatty acids (PUFA). Insects are also rich in several micronutrients: copper, iron, magnesium, manganese, phosphorous, selenium, zinc, riboflavin, pantothenic acid, biotin, and in some cases folic acid (Rumpold & Schülter, 2013). Due to low consumer acceptance of insects in European countries, edible insects may require processing into palatable dishes. Protein extracts or isolates may then also be an option.

#### 3.4.4 Single cell proteins

Single cell microorganism are characterised by a high protein content (40-80% w/w of dry cell weight), and are therefore considered single cell proteins (SCP). The quality of the protein is high and resembles that of animal origin, making the protein readily bioavailable. Other advantages of SCP are: rapid succession of generations (algae, 2–6 h; yeast, 1–3 h; bacteria, 0.5–2 h); broad range of substrate (carbon sources); continuous production with consistent quality; independence of environmental conditions; low land requirements and ecologically beneficial.

Research on single cell protein has seen a recent revival. In the seventies, dried bacteria were produced as feed and sold under the name Pruteen. However, this could not compete to soy on a price level. With rising soy prices, and with sufficient consumer acceptance is considered an option again. Regarding nutritional quality of SCP, protein represents about 80% of total microbial nitrogen, and contains all essential amino acids. Fungal SCP compares well with egg albumin, except in sulphur containing amino acids, which are deficient in this SCP. Formulation of fungal SCP together with other protein sources can balance the excess of nucleic acid as well as the deficiency on sulphur containing amino acids. Other main SCP include yeast that is commonly used in poultry food formulations and more recently, in feed mixes for fish (Ugalde & Castrillo, 2002).

# 4 Protein processing technology

In 2013 the STW research programme started with five technology projects on sustainable protein recovery, which aimed at developing innovative methods to extract proteins from plant leaves, microalgae and insects to meet the increasing demand for food proteins for humans and livestock.

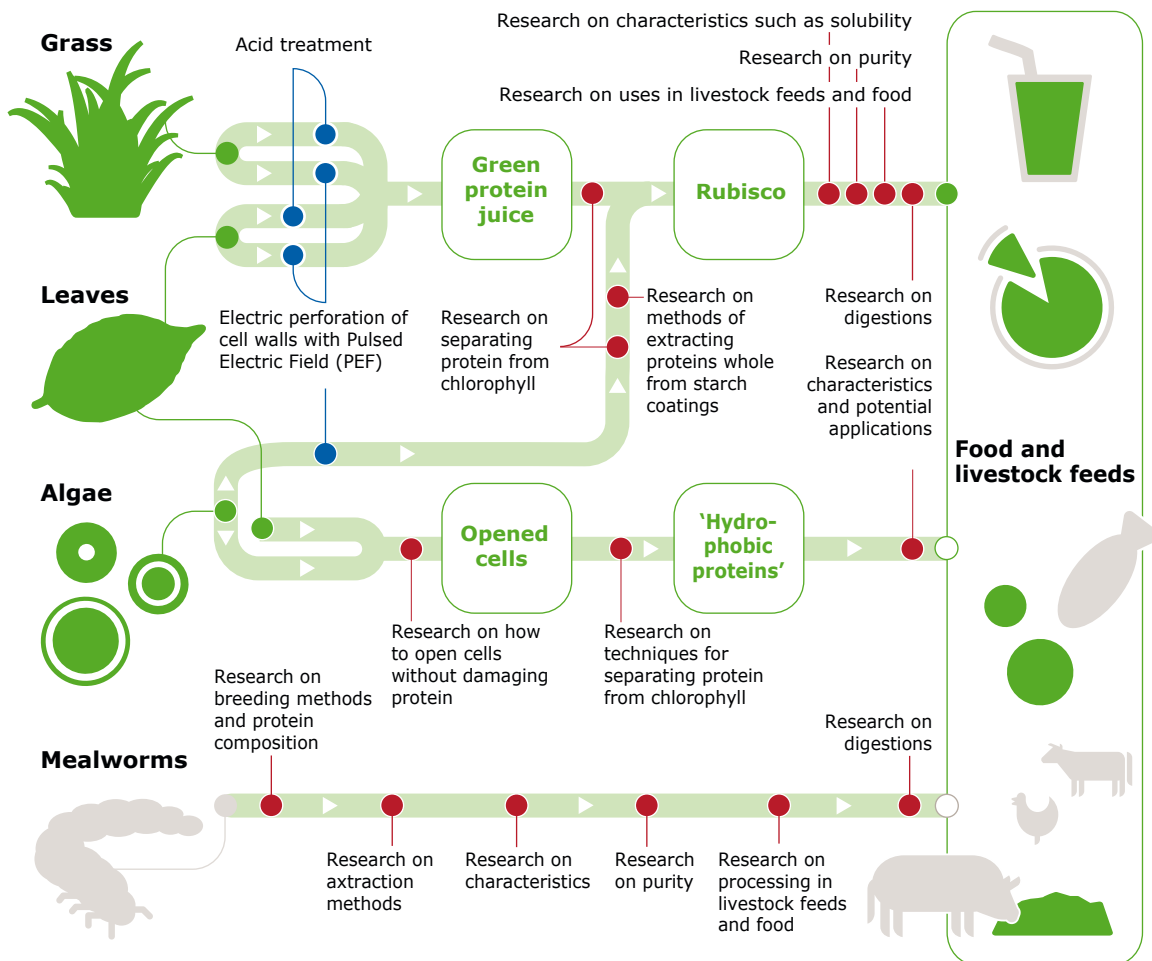


Figure 4: Research within STW protein Programme, depicted at the start of the projects (picture from Wageningen world, volume 1, 2013)

The following chapters describe important generic achievements of the five projects, as well as the areas that require further research and development. These challenges are described

below. Steps forward were achieved within the projects, but further development is still required for most technologies.

## 4.1 Improving extraction: PEF

Several of the projects saw a relatively low yield of protein, caused by poorly accessible cellular material. Pulse Electric Field (PEF) was suggested as a method to open up the cells, enabling easier extraction of protein. The projects gained experience with PEF technology for grass and algae. Both extractions were only very limited in their success. This is due to the underlying mechanism of PEF, which disturbs cell membranes, but leaves the cell wall intact. As cell walls are present in plants and most algae, proteins from these types of biomass are still hindered during extraction. Previous successful studies had either attempted to extract protein from sources without a cell wall, or had extracted much smaller molecules like monomeric sugars. A better combined understanding of plant physiology and PEF technology would have given faster results within the projects. Based on these learnings, follow-up projects within Wageningen now show good results with PEF-systems that aim for extraction of other components.

## 4.2 Improving extraction: cell wall degradation or disruption

A common denominator in all plant related projects was that the cell wall occurred to be one of the major hindrances for protein extraction, as proteins can't pass them. We briefly describe some

of the most popular methods for protein extraction. Each method has inherent advantages and disadvantages. As a rule of thumb the more disruptive methods can reduce extract viscosity but can also result in the inactivation of labile proteins while gentle treatments may not release the target protein from the cells, and resulting extracts are extremely viscous. Viscosity development in the extract is caused by cell debris (e.g. fibre) and affects the kinetics of further chemical and enzymatic extraction methods. Moreover, extremely viscous extracts are difficult to handle (e.g. clogging of machinery) and require big amounts of energy to be processed. A lot of effort has been put over the last years for developing methods to complement established disruption procedures and accomplish these tasks with even better yields (Grabski, 2009).

### 4.2.1 Mechanical cell wall degradation

Different mechanical methods are applied for protein extraction through disrupting cells. The main principles of the techniques used (1) to shearing by liquid flow, (2) to exploding by pressure differences between inside and outside of cell, (3) to collision forces by impact of beads or paddles, or (4) a combination of these forces. Table 1 describes suitability of the techniques to certain biomaterials (Goldberg et al., 2008).

Table 1 Physical techniques for cell disruption and their applicability to different biomaterials.(table adapted from Goldberg et al., 2008)

Technique	Type of biomaterial to be lysed			
	Bacteria	Yeast, Algae, Fungus, Spores	Seeds	Plants
Bead impact – shaking vessel	Y	Y	Y	Y
Bead impact – agitator shaft	Y	Y	?	Y
Rotor/stator – shear by spinning shaft	N	N	Y	Y
Mortar/pestle – shear by mechanical pressure	Y	Y	Y	Y
High-pressure batch – liquid expansion	Y	Y	?	?
High-pressure batch – gas expansion	Y	N	N	Y
High-pressure flow – high velocity shear	Y	Y	N	N
High-pressure flow – opposed liquid streams	Y	Y	N	N
Droplet – low pressure droplet nebulizing	Y	Y	N	N
Ultrasonic – shear collapsing bubbles	Y	Y	N	Y
Electro water separation	N	Y	N	N

Suitability: Y—general good practice; N—not recommended; ?—not known or marginal success

#### 4.2.2 (Bio)Chemical cell degradation

Alternatively, cell walls can be disrupted by (bio) chemical degradation. Alkali amount (Zhang et al., 2014) or pH (Sari et al., 2015) are the most important factors determining protein extraction yield. The influence of pH on protein extraction may occur through two mechanisms, by altering cell wall and protein properties. Disruption of cell wall surface properties involves a reduction in surface tension, partial removal of lignin, and complete removal of acetyl or uranic esters group of hemicellulose (Ashori et al., 2012). Also crystallinity of cellulose can be reduced following alkaline treatment (Mittal et al., 2011). Next to that it increases protein solubility that is lowest at the protein iso-electric point (around pH 4.5) but increases with increased pH.

Next to that, enzymes like proteases or carbohydrases can be used to increase extractability. Proteases lead to a reduction in protein size through proteolysis yielding (poly) peptides, while carbohydrases break down the cell wall. In both cases the proteins can be more easily extracted. Highest increases in protein/peptide yields are observed when proteases are used, rather than carbohydrases (Sari, et al., 2015) again illustrating the difficulty of breaking down the cell wall.

#### 4.3 Browning

Protein fractions from leafy sources, but also from insects, tend to brown rapidly. They can be therefore characterized as poor sources of native proteins. The brown pigments occur due to cross-linked protein with oxidised phenolics (Pierpoint, 2004). This chemical process resulting to this characteristic colour is known as enzymatic browning' (Ozidal et al., 2013). The phenolic compounds react with the protein especially under oxidative stress, leading many times to the changes in protein properties such as structure, solubility, digestibility and thermal stability (Chiesa & Gnansounou, 2011; Ozidal et al., 2013). This enzymatic reaction is difficult to avoid, since the variety of phenolic compounds in the different biomasses makes it difficult to suggest a specific protein extraction procedure. Prevention can be found in the addition of

antioxidants such as ascorbic acid or sulfites, or in the removal of oxygen during processing.

#### 4.4 Pre-treatment: Ethanol extraction

Protein extraction may result in lower product quality when other, unwanted, components are co-extracted. This was observed in almost all of the projects when polyphenols co-extracted with proteins as was explained in the previous paragraph. Polyphenols occur in leaves and insects and can be oxidized to brown pigments.

Within the grass research project the pre-extraction of leaves with ethanol was tested to remove toxins that might be co-extracted from weeds. A similar process might be developed for polyphenols, as they are soluble above 50% ethanol. Ethanol extraction could then be a valuable pre-treatment before protein extraction, which can be combined with removal and/or extraction of other components, such as colour and fragrance, but also of principal components such as sugars and organic acids.

#### 4.5 Fractionation instead of purification

Fractionation of biomass into functional fractions is an interesting concept that enables full use of raw materials. Instead of aiming for molecular purity, processes follow a functionality-driven approach, which has been successfully applied on lupine seed, oil-rich legumes, pulses, sugar beet leaves, and non-food crops like rubber-seed kernel. Less refined fractions are produced through mild fractionation, either by avoiding extreme temperature or pHs, or by reducing the use of water (e.g. dry fractionation and electrostatic separation). The resulting fractions are assessed in terms of nutritional value, functional properties and molecular purity. Depending on the biomass, special attention must be given to high levels of antinutritional components, which might remain in the products due to less processing.

Understanding the raw materials is beneficial for the development of fractionation processes. This understanding goes beyond compositional characterisation, and considers the anatomy of the biomass, its original function in the plant, the interactions of component and the level at which



these interactions take place. For example oil seed, pulses and cereals are characterised by organelles where components are stored/accumulated. Oil bodies (0.5 – 5  $\mu\text{m}$ ) and protein bodies (< 3  $\mu\text{m}$ ) are typically found in seeds, while starch granules (10 – 100  $\mu\text{m}$ ) are accumulated in roots and grains. For such raw materials, dry fractionation can separate these organelles based on particle size, resulting in enriched products. In contrast, leaves and aquatic plants contain most of the proteins in the chloroplast (3 – 5  $\mu\text{m}$ ). Inside this organelles RuBisCo is in solution, while membrane proteins are strongly attached to pigments and lipids forming the thylakoid membranes. These examples show the diversity of proteins microstructure and interactions in different sources of biomass. An a priori knowledge of physiology of biomass provides valuable routes for targeted protein extraction and therefore increase of purity and yield of the extract.

#### 4.6 Modified proteins for functionality

Hydrolysis of protein isolates under controlled conditions has shown positive effects on protein functionality. Hydrolysed protein has a remarkably higher solubility and is therefore considered high value protein with application areas ranging from food, feed, pharma and diagnostics. For instance, hydrolysis up to 10% of pea protein isolate

improved solubility, foaming and emulsifying properties (Barač et al., 2015). Protein hydrolysis is also used for the production of bioactive peptides from protein isolates and concentrates. These peptides are then considered as nutraceuticals and are valuable due to properties such as anti-hypertension, antioxidant, antimicrobial and antithrombotic activities. These peptides can be derived from soy protein, white protein fraction from alfalfa leaves, amaranth protein, insects (silk-worm) and protein from spent grains.

Another common way to modify proteins for novel functionality is through Maillard reaction. Maillard reaction constitute an efficient method to improve functional properties of proteins, such as emulsifying properties, heat stability, and antioxidant activity. Maillard reaction is a non-enzymatic reaction between amino groups of proteins and reducing ends of carbohydrates, which develops into a set of complex reactions. For instance, recent studies show modification of soy protein isolate and canola protein isolate through Maillard-reactions using soy hulls and gum Arabic, respectively. In both cases, better and even superior functional properties were observed (Wang et al., 2017; Pirestani et al., 2017).

# 5 Protein Product and application

Agricultural products can contain substantial amounts of proteins. A number of proteins both derived from plants and animals, have been produced commercially for a long time. Traditionally, food and feed proteins can be found in different types of biomass; in cereals (such as gluten in wheat and zein in corn), legumes (such as pea), oil crops (such as soy seeds, sunflower seeds), in dairy products (such as casein in milk), meat, fish and tubers (such as potato).

By far, the largest amount of proteins is being used in feed, followed by food consumption. Only a limited amount of proteins is isolated for specific use, for example as emulsifiers, in several food formulations, while even smaller amounts are valorised for industrial applications (chemicals, materials).

However, next to uses of proteins in the food and feed sector, proteins can also be used in more technical biobased applications, which is the current suggestion for new protein sources and protein rich residues produced by the agro-industrial sector. The restriction on the use of extracted and isolated proteins from such sources for food and feed applications lies in the fact that these "new" proteins have to pass the Novel Food regulation law. As long procedures can be foreseen, the outlet of new proteins in bio-based applications could be more feasible in the short term. Taking into account the search for more sustainable biomass use in biorefinery processes, newly exploited streams which fit this category, will become available in the near future. Applications that derive from the characteristic physicochemical properties of the proteins, such as thermal stability, solubility and structure are their use as binders in coatings and adhesives, as surface active agents and as green chemicals. An example of such protein is *Jatropha* protein, which has been excluded from food consumption (Sari, 2015).

Besides native proteins, (oligo)peptides such as hydrolysates have potential in several end

application. Protein hydrolysates have applications in specialty foods in e.g. sports, infant and medical nutrition. Hydrolysis is also needed to obtain protein structures with surface active (such as emulsifying) properties. Therefore, protein hydrolysates can, for example, also be used in cosmetic products such as shampoos, creams and coating systems.

Protein processes and their products can be characterised based on yield, purity and product functionality. This all relate to processing cost and product prices. Correlations between these factors can be different for different biomass sources, as we illustrate in the following paragraphs by comparing different clusters of plant protein and proteins from animal origin (Sari, 2015).

## 5.1 Biomass sources and the yield-purity dilemma

Protein extracted from novel sources can be compared to currently applied plant protein sources in terms of extraction yields and purity of the final product. For this comparison, information on the purities of extracted proteins as a function of their extraction yields was compiled (Figure 5) for several crops.

Soy is considered as state of the art for protein extraction, given the massive scale of its production and application. Lupine, rapeseed and some pulses are relatively novel protein sources with similar or potentially similar performance as



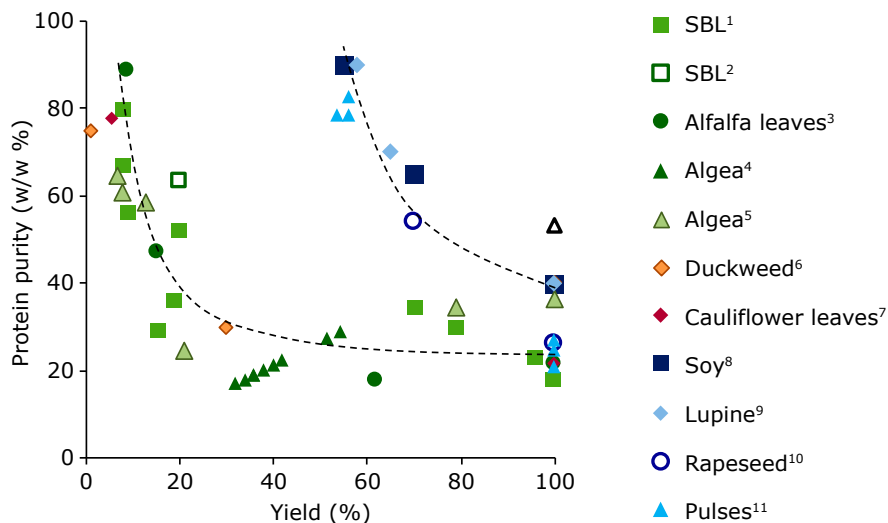


Figure 5. Yield of extracted protein as a function of the protein purity (w/w%, dry basis) (g protein in product per gram of protein in raw material). 1 (Tamayo Tenorio et al., 2017), 2 (Kiskini et al., 2016), 3 (Edwards et al., 1975), 4 (Postma et al.2015), 5 (Schwenzfeier et al., 2011), 6 (van Krimpen et al., 2013), 7 (Xu et al.2016), 8 (Cambell, 2010; Day, 2013), 9 (Berghout et al., 2015), 10 (Dijkstra et al., 2003), 11 (Boye et al., 2010). The point (Δ) highlights the high protein content of microalgae, known as an unconventional protein source (Spolaore et al., 2006).

soy. These protein crops follow a similar trend and establish processing goals of 50 - 60% yield with a protein purity of ~90%.

A different trend is found when considering protein yield and purity for leaves and other photosynthetic active tissues like algae and duckweed. The protein products are obtained at much lower yields. For these non-protein crops, a high-protein extract (60 – 80 w/w% protein) is only achieved at less than 10% protein yield.

Among the reasons for this large difference in yield are the type of proteins present in these crops (e.g. storage proteins vs. enzymes), the dry matter content (85 - 90% in protein crops, 5 - 15% photosynthetic crops), and possibly the lack of technology to process the wet photosynthetic active tissues. The technology to be developed must bear in mind the high level of organisation of the proteins in photosynthetic tissues, which occurs at small length scales and implies that larger driving forces and energy inputs are needed for separation. Attempts to new technologies include crystallisation, aqueous two phase systems, and pulsed electric fields, which still require further development before feasible implementation or may be only suitable for biomolecules different from proteins (Azmir et al., 2013; Baiano, 2014).

## 5.2 Functionality of protein products

Several factors determine the functionality of protein products: protein composition, shape, conformation; extraction and application conditions like temperature, pH, ionic strength and solvents; and processing steps towards the final product. Table 2 summarises the techno-functional properties of proteins, the major microscopic mechanism behind the techno functional property and examples of food and non-food applications. Interest in high protein diets is increasing as evidence mounts for their enhanced health benefits and environmental sustainability. However, plant proteins vary in their ability to provide all of the essential amino acids in amounts needed to meet human requirements. Soy protein so far is the only widely available plant protein that has been shown to be similar with animal proteins based on numerous nitrogen balance studies using isolated soy protein (ISP) or soy protein concentrate (SPC). The protein digestibility-corrected amino acid score (PDCAAS) is the most widely recognized and approved method for evaluating protein quality of food proteins (Hughes et al., 2011). PDCAAS is required by the United States Food and Drug Administration (US-FDA) labelling regulations, when making claims about protein content. The method is based on comparing the amino acid profile of a food protein to a reference value to determine an amino acid score. The amino acid score is corrected through taking into account the digestibility of the protein, resulting in a PDCAAS



value. On the basis of the rationale of the method, a PDCAAS value of 1.00 or 100% would indicate that a protein provides adequate amounts of all of the essential amino acids, when the protein is fed in nutritionally appropriate amounts, to children age two and above and adults. While most plant proteins, such as pea protein concentrate and other legumes, have lower PDCAAS values than animal proteins, soy protein has been shown to be comparable to milk, meat, and eggs (Hughes et al., 2011).

Moreover, besides the source of proteins (once the biomass is selected) different processing conditions are used to tune the final properties, which depend on the desired product. This can have huge effects in the bioavailability of proteins as well as on their techno-functional properties. For instance, protein isolates from red lentil showed almost twice higher fat adsorption when using ultrafiltration (115 g oil/100g material) compared to isoelectric precipitation (226 g oil/100g material) to obtain the isolate (Boye et al, 2010). Similarly, protein isolate from corn germ had four times higher water holding capacity (WHC) when tested at pH 7 (WHC 9 ml/g), compared to pH 10 ((WHC 2.2 ml/g) (Hojilla-

Evangelista, 2012). The pH-dependence of functional properties is especially observed with solubility, and the final pH conditions can affect the final solubility more than the protein extractions conditions. Applications such as foams and emulsion demand high protein solubility. In contrast, high solubility of the protein is not the determinant factor for meat analogue applications. Instead, in such products, proteins are required to have good fat and water absorption, as well as emulsifying and gelation capacity, and appealing sensory attributes (Barać et al., 2015).

From our discussion above and the previous section (e.g. figure 5) it is clear that obtaining high protein fractions through the current extraction methods requires a lot of energy and cost(due to low yield). The techno-functional characteristics of high and low purity fractions can differ based on the method and also the source of which the proteins are extracted. Often non-protein compounds present in the fraction are not easy to handle, can lower the reproducibility and cause destabilization of a complex food formulation. Moreover recent consumer interest in high protein diets makes protein isolates as standalone product in the market.

Table 2. Techno-functional properties of proteins in food and non-food systems. Adapted from Kinsella et al., 1982.

	<b>Techno-functional property</b>	<b>Mode of action</b>	<b>Food system</b>	<b>Non-food systems</b>
<b>High solubility</b>	Solubility	Protein solvation	Beverages	
	Emulsification	Formation and stabilisation of emulsions (W/O - margarine, O/W - mayonnaise)	Sausages, sauces, soups, cakes, salad dressings, ice-cream, yogurt	Surfactants in cosmetics and shampoo, wetting agents
	Foaming	Formation and stabilisation of foams	Whipped toppings, desserts, cakes	
	Gelation	Protein matrix formation and setting	Meats, curds, cheese, meat analogues	
<b>Intermediate solubility</b>	Cohesions-adhesion	Adhesive material	Meats, sausages, baked goods, pasta	Paper binder, bone glue
	Elasticity	Hydrophobic binding in gluten, sulphide links in gels, ion-mediated gels	Meats, bakery, cheese	Bio-plastics
	Viscosity	Thickening, water binding	Soups, gravies, low-fat products	
<b>Low solubility</b>	Fat adsorption	Binding of free fat	Meats, sausages, cakes, bakery	
	Flavour binding	Adsorption, entrapment, release	Meat analogues, bakery	Pharmaceuticals
	Hydrophobic films	Interfacial network formation	Food coatings	Paint, ink, paper/ packaging coating, bio-plastics

Despite that, the recent trend for natural and mildly processed food products has led a lot of research to study the techno-functional characteristics of non-purified protein fractions. The general trend is that isolates and in general higher purified fractions can have better functionality than less purified fractions. Nonetheless there is plethora of examples available that synergistic effects between the proteins and the rest of the components can be present improving existing or even providing novel techno functional characteristics (Geerts et. al, 2017; van der Goot et al., 2016).

### 5.3 Protein product functionality and price

For food and feed applications, “new” potential protein sources are several leaf proteins (grass, beet leaves), legumes (peas, lucerne), grains (amaranth, quinoa), aquatic biomass (seaweed and micro algae), insects (mealworm) and single cell proteins (fungi, bacteria, yeast). Plant proteins could provide new and sustainable food ingredients for humans to meet the growing demand for protein. However, when calculating future profits for the protein transition it is important to realize that plant proteins in general have lower prices compared to proteins derived from animal sources.

Table 3 presents an overview of products that are commercially available, including novel sources such as microalgae and insect powders. In general, the prices of animal proteins are double the prices of plant proteins. For example, the prices of whey protein (80 wt% protein) and casein (90 wt% protein) are twice as high as the price of a soy protein with a comparable protein content. This is on one hand caused by the excellent functionality and on the other hand by their traditional use in food products. For the novel sources like microalgae, insect and duckweed (table 3), the products are commercialised based on their nutritional and nutraceutical value, without information on their techno-functionality. Therefore, the high market prices might reflect a small market size, lack of technology or lack of large scale production.

For products with a relatively low protein content (35-50 wt%), such as flours, the price is about 1 €/kg. For the others, prices vary between 2 and 3 €/kg. Plant proteins, however, are still limitedly used in food products, compared to animal proteins. This is mainly due to their lower nutritional value. This is also visible in feed where there is a direct correlation between protein content and price of products (Figure 6). The presence of anti-nutritional factors (ANFs) directly lowers product prices of e.g. rapeseed meal and sunflower seed meal, as the applicability is much

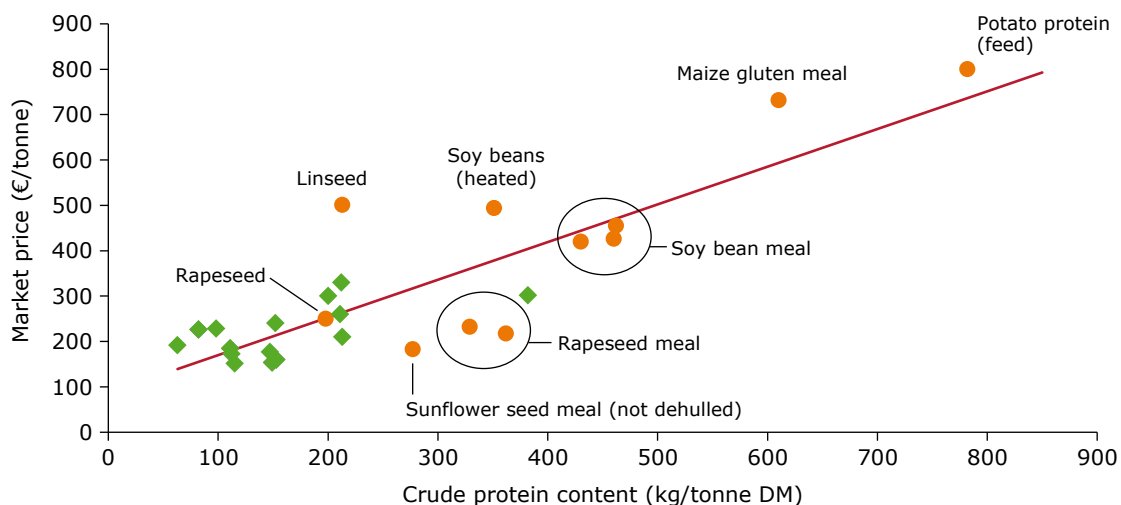


Figure 6 Correlation between protein content and feed price. Points below the line are lower because of the presence of anti-nutritional factors in the product, thus limiting its applicability (Teekens et al., 2016).

Table 3 Prices of protein products.

Plant-based proteins (protein content, wt%)	Price [€/kg]	Animal-based proteins (protein content, wt%)	Price [€/kg]
Soy meal (40%) <sup>1,2</sup>	0.4 - 0.8	Gelatine (low quality) <sup>1</sup>	2
Soy concentrate (>60%) <sup>3</sup>	1.2 - 2.8	Gelatine (high quality, pig, bovine, bones) (>80 wt%) <sup>1</sup>	4 - 6
Soy isolate (>90%) <sup>3</sup>	2.2 - 6.5	Gelatine (high quality, fish meal) (>90%) <sup>2</sup>	5 - 8
Pea concentrate <sup>1</sup>	2	Collagen-fish meal (>90%) <sup>2</sup>	15 - 20
Pea isolate <sup>1</sup>	3	Collagen - bovine (>90%) <sup>1</sup>	4 - 6
Lupine concentrate (55%) <sup>1</sup>	2 - 4	Egg white powder (>46%) <sup>1</sup>	6 - 8
Potato protein (feed) <sup>1</sup>	0.8	Egg yolk powder <sup>1</sup>	4 - 6
Potato protein -food (70%) <sup>1</sup>	>>3	Plasma powder <sup>1</sup>	3.5 - 4.5
Wheat gluten (84%) <sup>1</sup>	0.8 - 1.2	Haemoglobin powder <sup>1</sup>	0.7 - 1
Maize gluten feed <sup>1</sup>	0.12	Milk powder <sup>1</sup>	2.2
Zein <sup>1</sup>	25	Whey concentrate (30 wt%) <sup>1</sup>	1.6
		Whey isolate (80 wt%) <sup>1</sup>	5.5
		Casein/caseinate <sup>1</sup>	6.5
Novel sources (protein content, wt%)	Price [€/kg]	Novel sources (protein content, wt%)	Price [€/kg]
Amaranth flour (27%) <sup>2</sup>	4.3 - 4.8	Cricket protein powder (68%) <sup>5</sup>	25
Amaranth concentrate (70-85%) <sup>2</sup>	3.4 - 9.2	Silkworm flour (55%) <sup>5</sup>	49
Broad bean extract (60 - 80%) <sup>2</sup>	20 - 35	Mealworm powder - feed (65%) <sup>2</sup>	1.2 - 1.2
Chickpea flour (22%) <sup>3</sup>	0.33 - 0.66	Grasshopper flour (>64%) <sup>2</sup>	10 - 15
Lentil protein isolate (80 - 90%) <sup>2</sup>	1 - 100	Single cell protein - yeast extract (43.5 - 49%) <sup>5</sup>	9 - 32
Rapeseed meal (20%) <sup>3</sup>	0.12 - 0.33		
Corn gluten meal (60%) <sup>4</sup>	0.5		
Yellow pea protein concentrate (53%) <sup>3</sup>	1 - 2		
Yellow pea protein isolate (>80%) <sup>3</sup>	1.9 - 5.0		
Spirulina powder (60 - 67%) <sup>2</sup>	3 - 27		
Spirulina tablets (~60%) <sup>2</sup>	7 - 13		
Chlorella powder (50 - 70%) <sup>2</sup>	9 - 67		
Chlorella tablets (50 - 55%) <sup>2</sup>	11 - 22		
Moringa leaf powder (24.3 - 27.5%) <sup>2</sup>	16 - 25		
Alfalfa powder (13 - 16%) <sup>2</sup>	5 - 14		
Water lentil protein concentrate (~60%) <sup>3</sup>	0.024 - 0.04		

<sup>1</sup> Mulder et al., 2016<sup>2</sup> www.alibaba.com<sup>3</sup> <http://www.blonkconsultants.nl/?lang=en><sup>4</sup> <http://agebb.missouri.edu/dairy/byprod/allcompanies.asp><sup>5</sup> <https://www.thailandunique.com/insect-bug-flour-powder><sup>6</sup> www.amazon.de

lower. ANFs can bind to nutrients, including proteins, and inhibit the complete absorption during digestion.

The higher the protein content, the higher the price. But besides the protein amount, amino acid composition and protein digestibility are other important factors to consider. The concentration of amino acids such as methionine, lysine, tryptophan and threonine, are usually lower in

plant-based sources. Thus, combination of plant-based foods are proposed to increase the overall protein quality of a meal (Chardigny & Walrand, 2016).

Lately ingredients and chemical distributors have tried to present a generalized picture on the functionality of their plant derived ingredients with respect to the price. Very recently a study was presented by Cornelius distributor on the 14<sup>th</sup>

Global Food Technology and innovation Summit, based on their business which was afterwards acknowledged by several ingredient distributors (e.g. Cargill). Market considerations and functionality of proteins beyond nutrition were compared with respect to functionality and price on finished food products. For food applications taste was identified as the driving functionality. Based on the findings of the report, for food and feed applications though, they concluded to an hierarchy of protein value based on their techno functional properties. Solubility was on the top of the list, followed by foaming, emulsification and ending with gelling as the function property of the lowest value.

Finally, they correlated the functionality or performance of common animal and plant proteins to their price.

The analysis provided that texture development coming from plant based sources was the least important techno functional property. In recent years though, in the market, there is a sharp demand for plant based meat analogues (plant

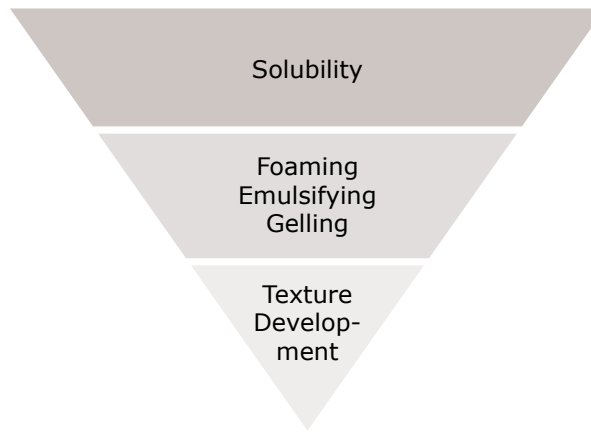


Figure 7. Techno functional hierarchy of protein functionality (picture adopted from Hart, 2015)

meat matters programme website; Wild et al., 2014) and the market projections for such products indicate even higher demand in the coming years. Based on this, we expect this characteristic techno functional property to increase its importance to the market and therefore increase the value of the proteins having the ability to develop texture (Wild et al., 2014).

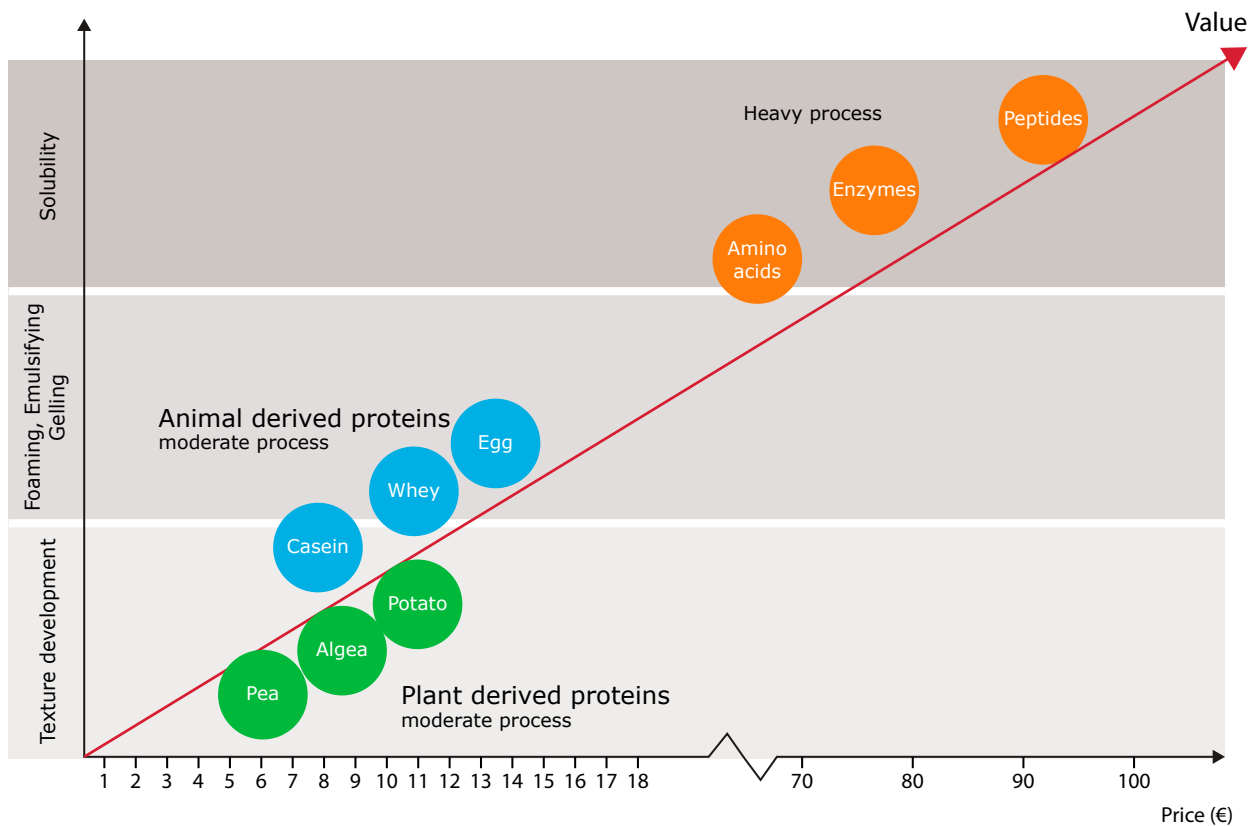


Figure 8 Functionality or performance of common animal and plant proteins to their price. (picture adopted from Hart, 2015)



# 6 STW protein projects evaluation

To evaluate the success of the protein programme, interviews were conducted with several of the people that were directly involved; both from university and from the research institutes. In addition, we asked for the opinion of relevant industries that were either directly involved in one of the projects or heavily involved in other protein related projects in Wageningen. This was done via a workshop. The input from both activities was also used for the final conclusions on future technological work on sustainable proteins. Projects content

The STW protein programme granted five projects aiming at the development and understanding of innovative methods to obtain proteins from sustainable/alternative sources of biomass. The sources of biomass were plant materials, micro-algae and insects. The main aim of the projects was to develop processes and applications that optimally utilize protein for applications in food and in feed industry.

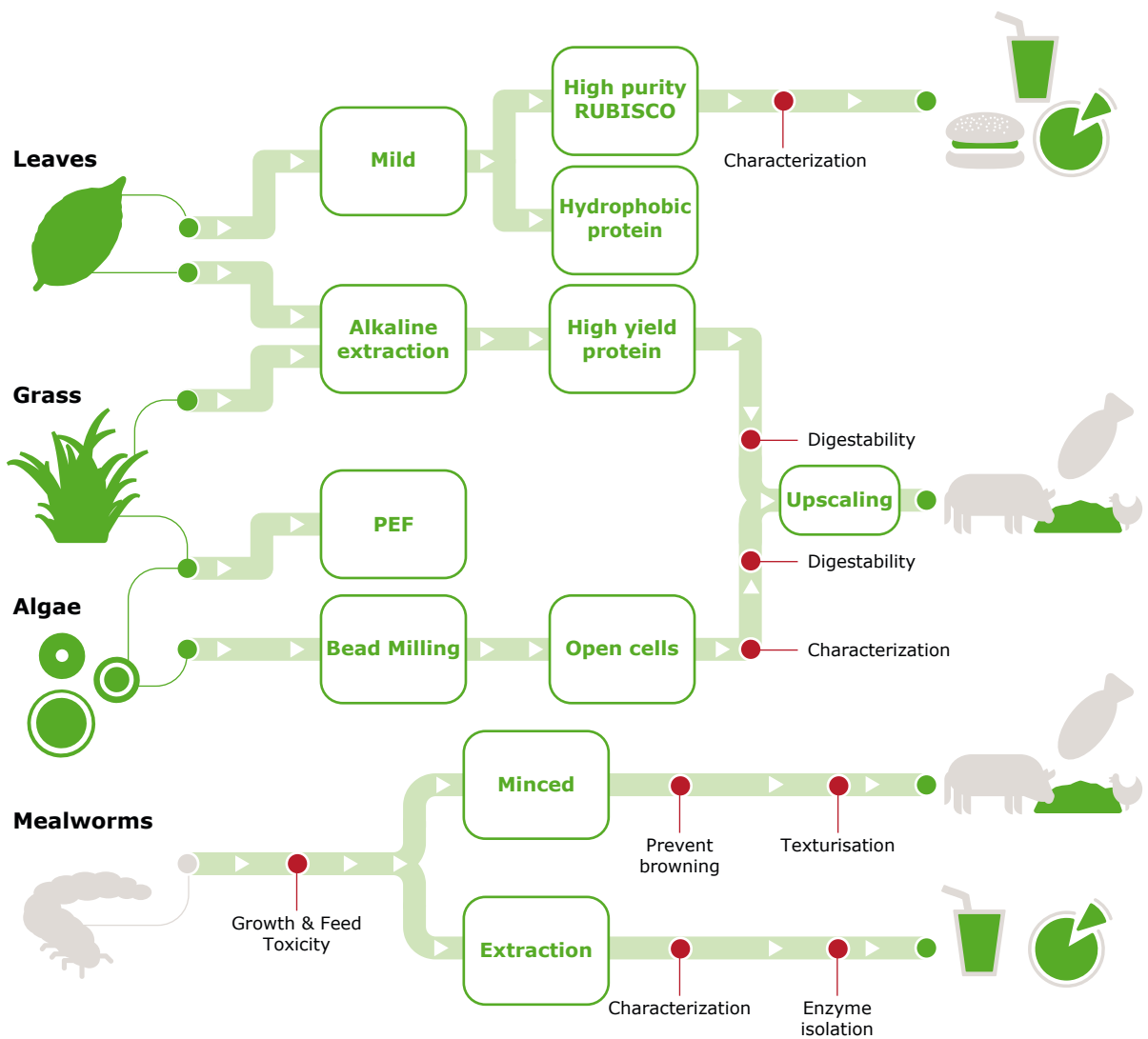
For the five projects common pillars of research approach could be identified:

- *Clever choice of biomass*  
By working closely with the industrial partners of each project, different sources of protein biomass were selected for investigation. The choice was primarily made based on source availability, protein availability, concentration of protein, and industrial relevance for food and feed applications. Other considerations were based on sustainability and cost.
- *Choice or development of different methods for protein recovery*  
Application of different traditional and alternative methods for protein extraction and development of new ones.
- *Results on protein yield/purity*  
Based on the extraction methods applied, protein yield and purity values of the fractions were determined with analytical techniques. The results were compared with protein extraction methods

on commonly applicable protein sources (e.g. crops, legumes, fish meal).

- *Characterization and techno-functional properties of protein-rich fractions, protein-rich fractions of low purity and all the other components present in the biomass.*  
The extracted fractions were characterized in terms of their composition and tested for typical functional properties such as solubility, foamability and emulsification properties.
- *Industrial applications – critical parameters for production; food and feed applications production cost and sustainability.*  
Working with the industrial partners of the project also directed the research on the applicability of the protein processes and products. Research results were evaluated in terms of industrial relevance, technologies were optimized, scaled-up and very often trials were performed in order to test the applicability of the findings on food and feed applications.

Depending on the research challenges the focus of each project was different. The applicability of each source for industrial applications is very much relevant to maturity of the research on the different types of biomass. Based on the research pillars described above, the PhD projects focused on different areas which were identified as the challenges with the higher impact on the research. Below the main area of focus is described for each project:



Schematic representation of the five research projects on extraction methods, processing, and applicability of alternative protein sources as conducted in the STW Protein Programme from 2013-2018.

In the *LEAP* project, available protein extraction methods were applied successfully on leafy biomass. The protein yield/purity though was identified as low. Therefore the main focus of the research was on the *development of new processes and applications* that utilize all the components particularly protein on leafy biomass. The research provided new methods for protein extraction with applicability in a wide range of wet biomass.

In the *GreenProtein* project different (available) extraction methods were applied to obtain high protein yield/purity fractions from green plant materials. The yield/purity fractions obtained were quite low and unsuitable for industrial applications. Therefore the research was focused on the

functional properties of non-pure protein fractions. The results showed very good *functional properties* for these fractions (non-pure protein fractions) with potential to even extended typical functional properties of proteins.

In the *In2Food* project the aim was the production and valorisation of high quality proteins from insects. While for this case the protein yield/purity fractions values obtained were good (similar values as protein extracted from soy) the quality of proteins was identified as non-acceptable for industrial applications. During the process side enzymatic reactions occur which leads to browning of the protein extract. Within this project the research was focused on the understanding and

ultimately prevention of *enzymatic browning* during extraction of proteins from insects.

The aim of the *Progress* project was to obtain protein-rich fractions from green sources. As green source different algae strains were tested and the protein yield/purity values obtained were similar and in good agreement with previous studies. Therefore the research was focused on the technological properties of proteins rich fractions. The aim was the fundamental *understanding of functional properties* at a molecular level and ultimately the manipulation of protein functionality for food and feed applications.

The *ALGAEPRO4YOU* research project was aimed at extraction and fractionation of functional properties from microalgae for potential application in the feed and food industry. Since the lab scale results showed huge potential towards this aim, the current focus of this project is on optimizing and providing theoretical framework for upscaling the process.

### 6.1 Conclusions interviews researchers

To draw further conclusions on the original aims and final execution of the STW programme, all project leaders, and PhDs were interviewed and asked for their opinion. PhD researchers were involved in the most of the workflow as described in the previous section. Therefore they got the overall multidisciplinary view of their project, of the whole programme but also on the higher purpose of their research. They were mostly involved in conducting in-depth research on the core challenges of their project, which provided the higher technological and scientific impact on the specific field. For that, they conducted experiments and reported their results either in closed meetings with the partners involved in their project or with the scientific community through conferences and papers. Their outcomes were identified as of high value and great technological and scientific importance. They found the contribution of the partners in the project very important; either through suggestions for their research and also by contributing actively in the project. They believed that the annual meetings of STW were a great opportunity to discuss their research, to find common scientific ground using

the perspective of the other projects, to obtain a broader overview of their research and to help each other. These meetings could have been held even more often.

In *general* all project leaders found the research of the programme interesting from both scientific and technological point of view as scientific excellence was combined with industrial relevance. The outcome so far provided *solutions* for key parameters with respect to *protein extraction from sustainable sources*. The scientific outcomes were developed for plant materials, micro-algae and insects but can be applied in principle for a wide range of protein sources. The technological results provide pragmatic solutions for implementation in industrial applications. All project leaders believed that the overall goal of the programme is a *multidisciplinary task* involving different parts of the chain. Working on that, they took into consideration all the aspects of the chain, identified potential risks and worked towards solving them. For this purpose continuous communications with the partners of the project was necessary. They found the involvement of the industrial partners of high value, and acknowledged their important contributions to the projects. Nevertheless, in a number of projects industrial commitment declined during the project, and at the end of the projects not all industrial partner were still actively contributing.

With respect to the *content* of the five PhD projects, all project leaders identified that it was *difficult to achieve protein extracts with yield/purity values, in a sustainable way*, similar to the values that can be obtained for crops or legumes (e.g. soy). Therefore, their research was tuned into identifying solutions on either developing new extraction methods or on studying non pure protein fractions for food and feed applications. That was achieved by investigating the *functionality of extracts, improving their quality* and identifying *potential synergistic effects* of proteins with the other components of the extract. Finally, all of project leaders agree and actually urge that further research is required in order to develop and implement innovative methods on proteins from sustainable sources for food for humans and livestock.



The STW call was unique in a sense that University groups and Research Institutes were invited to send in joint proposals. WFBR, TNO, Rikilt, and Livestock Research took part in the 5 STW protein projects. Lolke Sijsma (WFBR) and Aard de Jong (TNO) were asked about their experiences. Their comments are summarised in the remainder of this section.

Combination of University and Research Institutes worked well. This was not only given as their comment by the Research Institute, but also confirmed by the industrial partners. Industry was especially happy with the addition of Institute members to a PhD topic, because of their knowledge level, and the continuity in topics that is not the case with a solely PhD topic. Also the applied part of the developed scientific knowledge was better secured with this construction. On the other hand, Research Institute employees felt they had a good opportunity to go in-depth within the projects.

Although there were some administrative hurdles to be overcome, cooperation on the content was

well perceived by both university and research institute. The division of tasks or subjects within a project was often not the classical fundamental/applied division, but more based on the combination of expertise of the different groups. Exceptions were up-scaling and product application questions that were more often addressed by the applied groups. These allowed the projects to advance further into practical application of the results and to take economic impact into account, but also aided at the start of the project when a (relevant) type of biomass had to be chosen.

In future projects, the institutes would like intensified contact with especially the PhD students. The regular contact was appreciated and institute members co-authored some of the PhD articles. The collaboration between institutes and universities can be further intensified by acquiring more joint projects, either via EU or PPP projects, or via new joint initiatives like STW/EZ. The last year several follow-up project started, in which cooperation between the groups that participated in the STW programme was continued.



## 6.2 Industrial vision on research on protein technology

At July 4<sup>th</sup> 2017 a workshop was held to ask the opinion of industrial parties on the future of protein research. The total number of participants was 31 and they were first asked about their expectations of this workshop.



Only after that, they were given an introduction to (part of) the input that was generated within the current project through three presentations. Wim Mulder started by presenting about the “Biorefinery of plant biomass (based largely on Chapter 3)”, followed by Jan Peter Lesschen on “Options to improve protein efficiency in Dutch agriculture (paragraph 2.2)”, and Marieke Bruins with a “Summary of 5 STW-protein projects (Chapter 1 and Paragraph 6.1). Participants were then asked which areas they considered relevant for further investments in future protein research. Industry clearly thought that side stream valorisation was key, while researchers thought that a focus on alternative protein sources was most important.

The research areas that the participants could choose from were derived from the previous interview answers. In addition, the workshop participants were asked for additional areas that they found important. Combined, this led to a list of 7 areas that were voted on. The Top 3 were further discussed in small groups.

### Product driven research

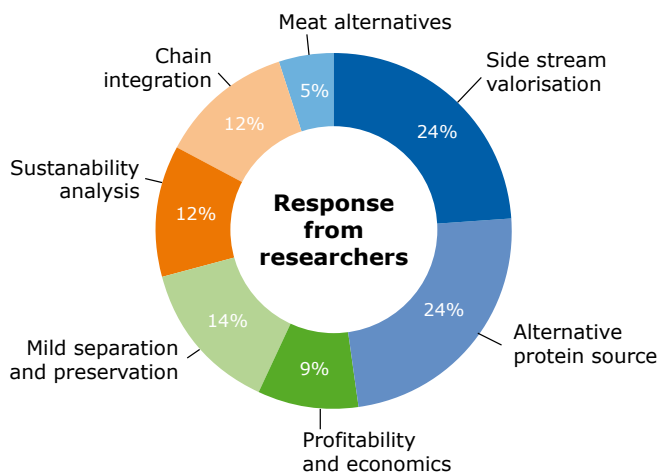
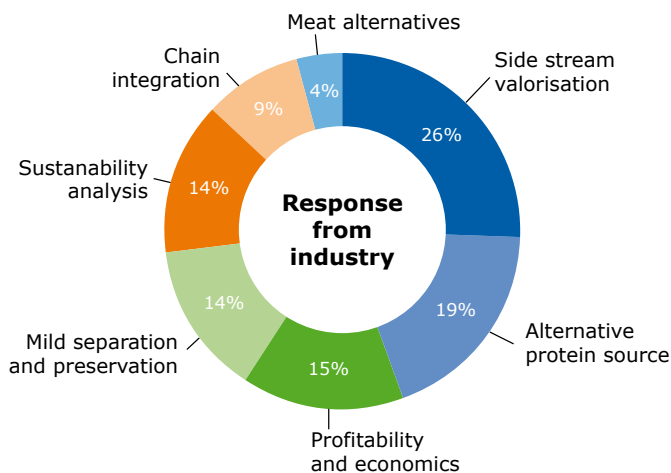
The desired end-product of a process determines requirements such as colour, taste, texture, functionality, digestibility, price, clean label, of the produced ingredient. The focus in research for food proteins should thus not be on obtaining a multi-purpose pure protein, but on obtaining a functional protein-rich fraction that is fit for a certain application. Variations in starting material should however still lead to robust product quality. It might require flexible processing, or aiming for a smaller outlet. The concept is in line with consumer attitude that is going to less refined products.

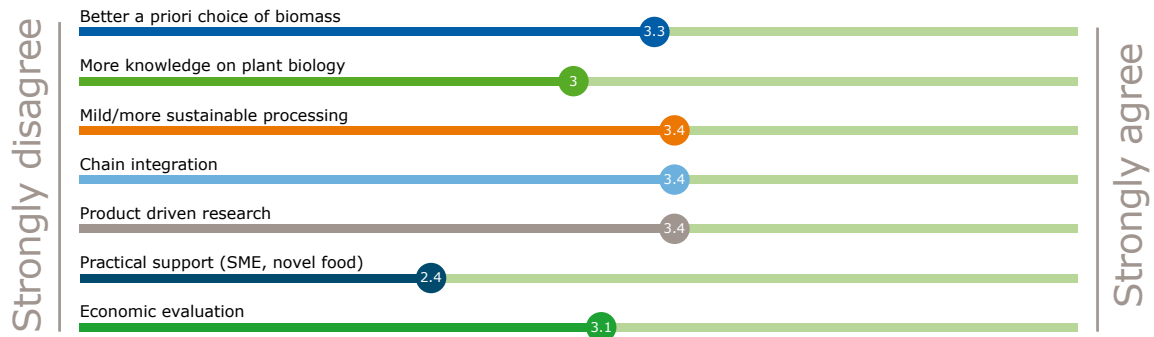
### Chain integration and side streams

Chain integration is important to achieve sustainable and economical feasible solutions. This incorporates topics like crop selection, crop rotation, integration with livestock, logistics, storage, processing, soil, and consumer perception.

Short term projects focus on the optimisation of existing chains. This is of direct interest to the industry. They are also interested in the development of new chains, with e.g. different

### Where should EZ put their money?





crops or different/new products. One crop can generate multiple outlets that were not utilized before. However, these developments are far more difficult to implement and ask for a long term approach (as in 10 years). This is not possible in current programmes that mostly deliver parts of the solution.

Research in this field should be on system optimisation, taking people, planet, and profit into account. Research questions can include: Can the Netherlands/Europe be self-sufficient for protein, and how is optimal (complete) valorisation achievable. It will be important to look at large sidestreams with potential value, waste reduction, and the integration between food/feed and non-food. System optimisation can aid in more effective use of nitrogen in general, and protein specifically.

#### Mild, sustainable processing

Mild processing can be key to more sustainable solutions. Processing should be selective to remove dysfunctional components and/or obtain functional fractions for direct application in food products. It is important to look at proteins in their final product matrix, and on the interaction with other ingredients. Preservation and stabilisation of the product should not be neglected during separation.

Development of mild separation can benefit from more knowledge on complexity in nature, more specifically plant physiology. Basic knowledge on physiology of plant tissues and their biochemical differences can aid the development of new technologies. It can also be a starting point for discussions and improvements in plant breeding.

Research should be on both old and new technologies, of which food fermentation was

specifically named as an example of an “old technology” with new possibilities. Less sustainable technologies like drying may be (partly) replaced by mechanical means of dewatering where a paste is produced instead of a powder. Also in this line of research, it is important to look beyond a certain unit operation and use a system approach, including crop selection and co-valorisation.

#### General remarks

During the workshop there were also a few more general comments that were not on new content.

- A multidisciplinary approach is necessary in these types of projects. Knowledge on technology needs to be combined with e.g. knowledge on plant physiology, component interactions, and product application.
- Senior researchers have to be involved to have a broad vision, which PhD students don't have at start of project. It will aid in a faster start-up in projects and lead to research which is embedded in a broader context.
- Increase in use of plant proteins is goal of government, not from industry. However, industry is very much willing to participate when an economic advantage can be combined with solving a societal problem.

Finally people were asked what they got out of the workshop. In general it was very inspiring and the workshop had given them insight in, and an overview of, current and future protein research.



# 7 Conclusions and recommendations for further research

The evaluation of the STW protein programme and the interviews with the scientific community and industry provided suggestions for future research. These suggestions were grouped and summarized leading to the following conclusions and recommendations.

With a growing world population and changing diets, the global demand for animal proteins for human consumption is expected to increase. However, the production of animal proteins has a significant impact on the environment, including expansion of agricultural land, greenhouse gas emissions, eutrophication of surface waters and nutrient imbalances. The inefficiencies originate from the fact that only part of protein in feed is retained in live weight and 5%–40% in the edible weight. Several options are available to improve this efficiency within agriculture and/or reduce waste, while a lot of effort is spent on innovations and technological improvements to increase efficient use of, specifically plant, protein for food. Most of the projects in the STW protein programme aimed for better using by-product streams. This could be achieved directly (leaves), following a transformation via insects, or through better use of available reactive nitrogen (algae).

In most of the projects, the researchers initially strived for high purity, and specialty applications as this can increase economic potential. However, it should be noted that the size of that market is small, and already taken by protein-ingredients that are (traditionally) considered as by-product (e.g. whey protein and soy protein). Results showed that current technologies do not allow high purity protein at sufficient yields. Therefore a number of projects, investigated whether less or milder fractionation could be a

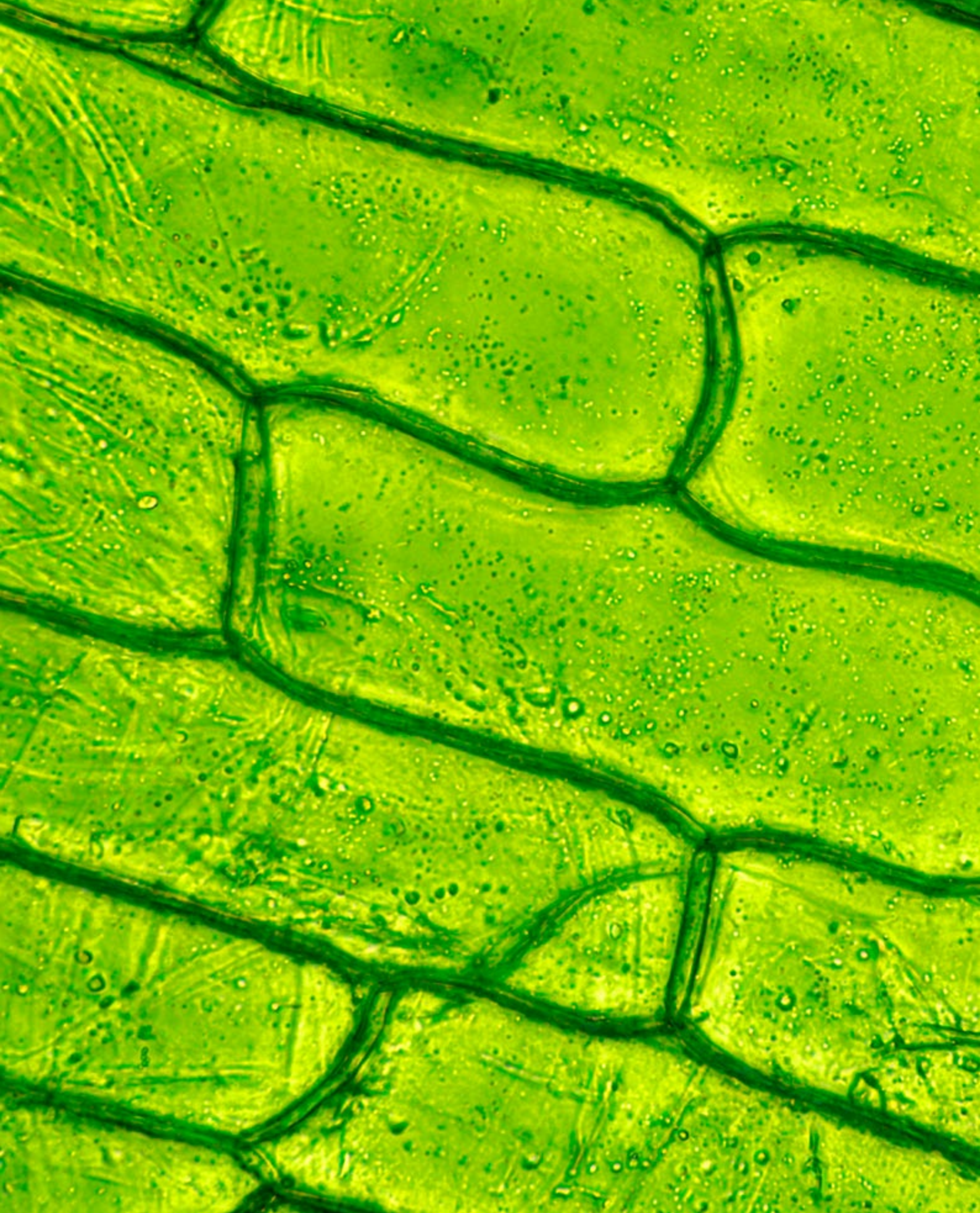
route to make so-called functional fractions. Those fractions are characterised by lower purity, but can have interesting properties that allow application in foods. The latter can be considered a key success factor for future projects and has already been a topic or research from industry.

## Approaches for future research

Research on protein technology aims at direct improvements in sustainability through development of new technologies, through development of entirely new systems that can have large impact, or through generating more fundamental knowledge on component interactions. All three approaches will aid in achieving the long-term goal of more sustainable protein use.

## Direct improvements by optimized use of traditional protein crops

Optimisation of current systems was indicated by industry as a means to facilitate quick wins in protein use efficiency. The focus on improvement of current systems, while using existing technologies in a new or better way, or by using innovative technologies would be of their interest in projects running for 2-4 years. Technology development was key in the previous STW protein projects, and aided to developments that can be relatively easily applied by industry.



### Comparing new green sources with traditional crops

A general conclusion from most of the projects was that a better a priori choice of the biomass under investigation could make research on biorefinery more efficient. Such choice on biomass should be based on criteria such as a) protein availability b) availability of current processing methods for protein extraction c) volume of the biomass and d) a priori knowledge of potential applications of the proteins and other fractions in a product. Better use of traditional crops through new technologies can lead to improvements that allow fast implementation.

For protein from green plant materials and from green sources in general, further investigation is required to significantly improve the protein purity and protein yields. It is important to gain more knowledge concerning the extraction of proteins from green fresh biomass in relation to conventional biomass. One aspect to involve is the biological architecture of the green biomass and the function of the different proteins in the biomass. Further, more knowledge is needed to be acquired on the type of protein, and the requirements in different end applications. The current bulk application for green proteins is often considered to be feed, but will find better use in the future as a direct source of protein in food.

### Whole biomass or protein products

When using protein from biomass, separation should not become a goal by itself. First, total use of protein-rich materials should be investigated and if possible for the main application, being solely nutritional. Animal feed can act as a benchmark application. For instance, in case of whole algae, the application in fish feed should be further investigated, given the oil content.. In several cases, nutritional quality requires removal of certain minor components, giving design rules for novel fractionation techniques.

Also other factors might limit total use of novel crops, especially for new protein-rich foods. For example, texturing properties are often not optimal. In that case fractionation can be applied. Initial focus should be on minimal processing, after which the properties of the fractions can be explored. Further fractionation, up to pure

fractions should be considered in case partially fractionated materials do not fulfil technical requirements. The purpose of pure fractions is twofold. Highly pure fractions can be tested for specialty applications, while mixtures of pure ingredients can be used to better understand the properties of multi-component functional fractions.

Sustainable and economical extraction of proteins at high purity and/or yield still needs technological improvement. This improvement can be obtained by steering away from high purity isolates, and focus more on functional fractions, in which the additional components might even have potential synergistic effects. Important scientific aspects are then a better understanding of the interactions between proteins and other components in plants, before, during and after extraction. This aids in improving current extraction techniques and in developing new ones

Also the functionality of the obtained extracts should be studied. Not only on a product scale, but also on a microscale, to better understand structure-function relationships. Aspects like colour and taste, and basic functionalities such as solubility should not be neglected, as most protein products are mainly used for their nutritional value, and not for e.g. their technological properties.

### Developing fundamental knowledge

Next to solving today's societal and environmental problems, science should aim at the development of more fundamental knowledge. Several aspects were already touched upon in the previous paragraph as more fundamental knowledge on component interaction is needed to develop processes to get protein out of complex biomass.

### Knowledge on plant morphology and cell biology

To better understand the fractionation behaviour of green material, plant sciences should be involved. For the separation of biomolecules from solid substrates, like leaves and algae, knowledge on biomass structure and component interactions is essential. As a basis, the in-situ interaction between the different components in their micro-environment (intrinsic factors) have to be unravelled. Questions like what is the (sub)cellular

and tissue location of components inside biomass have to be addressed, with emphasis on proteins, and the influence of tissue microstructure on their extractability. Besides, it should be investigated whether interactions can be reduced through plant breeding programs to facilitate fractionation, to alter nutritional profiles or reduce the content of undesired components.

### Developing new systems for large impact

Development of new systems for more effective use of protein, is seen as challenging and difficult to implement in actuality. It needs long term commitment that very often outlasts a 4 year project. However, it was indicated as essential when we really want to achieve major impact on sustainability. It is therefore highly recommended as a goal for integrated projects, as it is hard to achieve otherwise. Complete systems can also include research on logistics and consumer acceptance. Taking these aspects into account can lead to a faster implementation and thereby increase the impact of the performed research.

### Chain design

To get to large improvements, bigger steps need to be taken and whole new chains may need to be developed. New technologies can give leverage for such developments. New chains are needed when new biomass sources are used, or when new products are made. The use of entirely new biomass sources, like algae or insects, has many scientific challenges but also needs e.g. a legislation procedure (EFSA) in which solid scientific data can help. But new chains can also face the challenge of multiple products in different application areas, which is often the case in biorefinery where products are produced not only for food, but also for fuel, materials or chemicals.

### Process design

To complete the biorefinery concept, the (solid) residue obtained during protein extraction should also be used. The residue usage will reduce the overall processing costs for protein production, bringing protein biorefinery closer to industrial application. Although industry often likes to keep with existing technologies and processes, they might need to re-evaluate processes that previously focussed on other products like oil and

starch, and did not consider protein yield and functionality.

### Sustainability analysis

Sustainability is an important driver for the development of plant based protein products. It should therefore be an integral part of research. This will keep the focus on developing sustainable technologies and chains. Important aspects can be a limitation in processing steps, avoiding the use of (much) water, and the valorisation of all biomass components.

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# Appendix

## Summary of report of interviews for the 5 projects

### Increasing the utilization of low value leaves by use of new protein extraction methods - Biorefinery of leafy biomass using green tea residue as a model material (LEAP)

PhD (finished) Dr. Chen Zhang – Project leader Dr. Ir. Marieke E. Bruins – Thesis and interviews

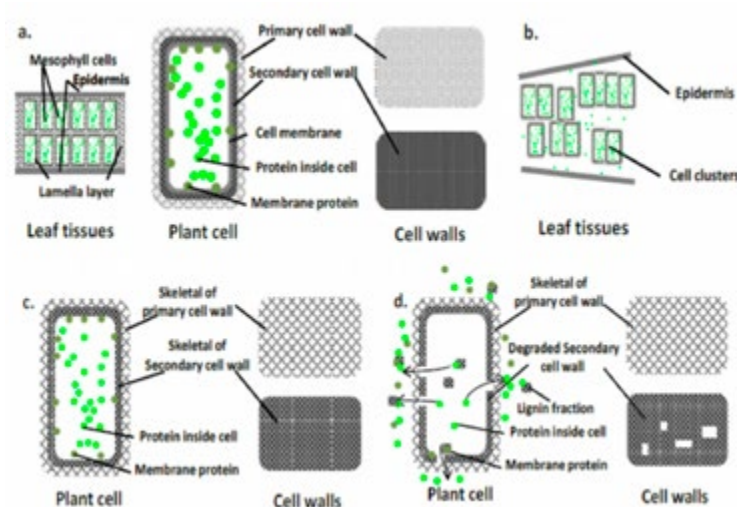
#### Summary of Thesis work

The aim of the LEAP study was to develop new processes and applications that optimally utilize all components, and particularly protein, of leafy biomass for application in the feed and/or food industry. A primarily method was developed and applied on green tea residues, which can also be applicable to other types of leafy biomass.

The research was focussed initially on the development of an alkaline protein extraction technology. In alkaline protein extraction, the temperature, amount of NaOH, and the extraction time are the parameters determining the protein yield, while the pH and the volume of the extraction liquid are critical parameters for the production cost.

Marieke think about the pic After optimization, more than 80-90% of the leaf's protein could be extracted by a single step of alkaline extraction at a cost of 102€·per ton-1 protein product. The extracted protein nutritional value was comparable to that of soybean meal and this technique can be adapted to various types of leafy biomass.

However, main drawbacks of this technique are the overuse of alkali, the generation of salts, and the destruction of key amino acids, such as lysine, during the extraction. In order to overcome these drawbacks, integrated processes were developed that recycled the chemicals and also aimed for additional products next to protein. During the project four different designs were made in which subsequent improvements were incorporated.



Hypothesized mechanism of alkaline protein extraction from leafy biomass on tissue, cell end cell wall level. a) simplified models of leaf tissues, cell structure, primary cell wall and secondary cell wall; b) first phase: solubilisation of lamella layer; c) second phase: solubilisation of lamella components in cell wall and cell membrane; d) third phase; disruption of secondary cell wall.



The first integrated biorefinery was based on the finding that alkaline protein extraction was not facilitated by increased solubility or hydrolysis of protein, but was positively correlated to leaf tissue disruption (see also figure). RGI (rhamnogalacturonan I) pectin and lignin yields were both linearly correlated to protein yield, which indicated that they are likely to be the key limitation to leaf protein extraction. At relatively neutral pH, HG (homogalacturonan) pectin, RGII (rhamnogalacturonan II) pectin, polyphenols, and organic acids can be extracted before protein. Protein extraction can be followed by the extraction of cellulose and hemi-cellulose.

Based on the above finding, it was evident that the cell wall was limiting protein extraction, therefore an integrated (second) biorefinery that combined protein extraction with a pre-treatment was proposed. Ethanol, viscozyme®L, and H<sub>2</sub>O<sub>2</sub> were selected as pre-treatments targeting on the removal of polyphenols and pigments, carbohydrates and lignin, accordingly. Ethanol and viscozyme®L could extract their targeting components efficiently, while H<sub>2</sub>O<sub>2</sub> could bleach GTR with no lignin extracted. The best pre-treatment was the combination of viscozyme®L and 50% ethanol extraction, which not only reduced the use of alkali by 50%, but also improved the protein content and its nutritional value.

After selecting pectin as a second product from leaves, different methods for its recovery were tested. As pectin can be applied for food or chemicals, mild conditions using enzyme and PBS buffer were investigated for pectin extraction for the integrated third biorefinery. *Both enzyme and PBS buffer extraction could extract high yield HG pectin (represented by galacturonic acid) without protein extraction, and also reduced alkali usage in subsequent protein extraction. Pectin obtained using PBS buffer could be present in its native form, after being precipitated by 40% ethanol. Buffer is suggested for pectin extraction when pectin is to be used in food. As an alternative, hydrolysed pectin that mainly contains galacturonic acid can be converted to useful chemicals. For this the enzymatic methods, enzymes such as Viscozyme®L, were recommended.*

*Alkali usage was then further minimized. It was found that by using potassium hydroxide, the protein extraction efficiency was similar to that using sodium hydroxide. The waste water from this process, mainly containing potassium salts, can then be used as fertilizer. This technique is highly dependent on the location of factories, which should be built close to the field.*

*Alternatively, calcium hydroxide can be used. As calcium salts can be precipitated by CO<sub>2</sub> and calcium hydroxide can be regenerated through burning of the precipitate, this scheme is sustainable and adaptable to most situations. However, as calcium can also precipitate pectin, polyphenols, and even proteins, the protein yield is relatively low. Although a pre-treatment can improve the extraction efficiency of calcium hydroxide, economic calculations suggested that a pre-treatment is not desired; unless the products obtained by pre-treatment have an attractive market value.*

Finally, the mechanism of alkaline protein extraction was explained in a simplified model of leaf tissues and cell walls (see figure). The model was also used to explain other mechanisms for protein extraction; mechanical milling, steam explosion, acid, and enzyme aided extraction. Future possible improvements of leaf biorefinery economics were illustrated as suggestions for the reduction of production cost, by e.g. using counter current extraction or ultrafiltration, or by upgrading product value by applying both protein and pectin in food. The processes recommended in this thesis show an excellent prospective, in which they are applicable to other leaf biomass and suitable for small-scale production.

#### Summary of discussion with Dr. Chen Zhang

The aim of the study was to develop new processes and applications that optimally utilize all components, particularly protein, of leafy biomass in the feed and or food industry. Green tea residue and grass biomass were used as systems of leafy biomass.

To Chen's opinion protein extract from leafy biomass is a good source for protein but the challenges and limitations lay mainly in its bad carbon digestibility. Carbon digestibility was measured by the RIKILT-WUR institute which was

partner in the project. Based on Chen's research, protein extract from leafy biomass, had a concentration of 30-40% and after purification, at yields that could reach values up to 90%. These values are quite high and show a great potential of protein extraction from leafy biomass. For practical applications though, the protein extracts still had a lot of impurities, including salts which makes the process quite costly and unsustainable.

Nonetheless Chen believes are that non purified (protein) extracts can have a great potential for food applications.

Important components which are present in the green tea extract:

- Pectin – extraction of pectin can be achieved with a yield reaching values of 80-90% ( e.g. through mild alkaline extraction)
- Celluloses – extraction of celluloses can be achieved with a yield reaching values of 25-30%

Towards the above approach, and further optimization of the protein biorefinery process of leafy biomass, his suggestions for future research concern:

- 1 Investigation on how extracted protein interacts with the co-extracted lignin.
- 2 Investigation on how the extraction methods (for protein and lignin) developed in this study can be applied to other types of biomass (e.g. algae) and study for any synergistic functionalities of the two components.
- 3 Identification of extracted solids and their potential use for food applications.
- 4 Investigate the functionality of extracted proteins (and lignins) for food products
- 5 Investigate how carbohydrase enzymes can affect the yield and the purity of extracted proteins, as alternative mild processing.
- 6 Development of models for better design of the extraction process from the beginning.

#### Summary of discussion with project leader Dr. Ir. Marieke E. Bruins

The aim of the project was to develop a method to obtain a protein rich fraction from leaves with application in food and feed. Leaves are a green

mainstream biomass of high nutritional value but often with no current function. The focus of the study was on better availability and upgraded sources.

The available biomass sources originally were: Cassava, sorghum leaves, grass and green tea leaves. In the beginning of the project, the study of all different sources of biomass was considered too broad. Therefore, it was decided to mainly focus to green tea leaves (done by the PhD). Experiments were also performed on grass biomass (by WFBR) to compare the two leafy systems. The biomass obtained from leaves is of high nutritional value, but challenges of the research were that the biomass was not always food grade (grass), its functionality was poor and the colour was considered not acceptable for neither food or feed applications. Having these challenges in mind, different methods were developed aiming for high purity and high yield protein extracts. Next to the improvement of the alkali extraction method, the highlights of the research include two major improvements which may be universally applicable for protein extraction from different biomass sources of proteins, potentially including alternative sources such as algae. These developments are:

- Pre-treatment of the biomass with ethanol. This was used to remove the brown colour, and remove toxins.
- Pulsed electric field (PEF) processing for protein extraction. This was applied and provided optimistic results. Further optimization is currently applied to test its potential for practical applications.

Besides these two technology highlights a new method was developed for integrated biorefinery of leaves which now serves as the basis for biorefinery of leaves. It was possible to obtain protein rich extracts that can be used in feed, but not with the desirable purity for some other practical applications. Therefore further research was not aimed on obtaining high purity protein components (e.g. further optimizing the methods) but focused on non-purified extracts with high protein yield. The non-purified extracts were characterized in terms of their composition and they were tested for their feed functionality. Also

all other fractions and residues of the process were partially characterized in terms of their composition.

Next to high scientific impact, the described research was aiming also to provide high impact for food and feed applications. However, from an application point though, there are several aspects which act as drawback for pragmatic applications; Proteins in leaves are not stable after the biomass has been harvested since side reactions are occurring during storage. As a result the fresh material is different than the stored material, which is a great obstacle for scaling up.

Besides the scientific and food/feed application, oriented findings emerged from this project on leafy biomass, the LEAP project provided more general learnings: A suggestion for projects on the biorefinery of proteins in general, is the a priori clever choice of biomass; this would require that the biomass should be selected in advance with

criteria such as a) protein availability b) availability of current processing methods for protein extraction c) volume of the biomass and d) a priori knowledge of potential applications and linkage to the choice of biomass.

Further research based on the LEAP topic has already started. This aims at the recovery of other valuable components from tea leaves (e.g. the recovery of polyphenols - a small spin off emerged that is the focus area of a PhD student).

Final remark on future valuable projects is on more detailed characterization of the functionality of extracts from all fractions of the process. This would include the functionality of high purity protein extracts and also the functionality of non-purified ones in order to probe the interactions of proteins with other (valuable) components.

## Isolation of hydrophobic proteins from green plant materials (GreenProtein)

*PhD (Finished) MSc Angelica Tamayo Tenorio - PhD defence 12th of May 2017, (Project leader: Prof. Dr.Ir. Atze Jan van der Goot)*

### Summary of Thesis work

Leaves have potential to become a source of protein and other valuable components. While isolated and purified soluble proteins (i.e. RuBisCo) are already considered for food production, there is added potential in leaf proteins that are currently discarded during RuBisCo isolation. The overall aim of the thesis was to obtain understanding on the use of green leaves as a food source by exploring currently neglected leaf fractions. The research was divided into three objectives concerning the extraction of membrane proteins from leaves, the properties of other valuable leaf components (complexes and fibres), and the feasibility of leaves as a food crop. Extraction processes of leaf proteins typically include a mechanical pressing step and subsequent thermal fractionation of the proteins. The heat treatment is thought to precipitate the

insoluble proteins together with fibres, chlorophyll and other components, resulting in a green curd that is discarded. Main results of the research are summarized below:

- Both *soluble and insoluble* protein distribute almost evenly over the leaf fractions: juice, pulp, supernatant and final pellet. The even distribution of the proteins is attributed to the anatomy of leaves and their biological function, which is predominantly the enzymatic activity related to photosynthesis.
- *Proteomic extraction* protocols were applied on obtaining *insoluble protein* fractions (isolation of membrane proteins). Harsh and/or non-food grade conditions were required to isolate the leaf membrane proteins with high purity. Although this process could be translated to a food grade extraction method, the final protein *yield*



achieved with leaves is much lower than for other plant sources that are already used at industrial scale (e.g. soy and lupine beans), suggesting the need for fractionation of the whole leaf, rather than fractionation of the leaf proteins (i.e. soluble and insoluble proteins).

- *Interfacial behaviour of thylakoid membranes* as stabilisers of emulsions. Thylakoid membranes were extracted from fresh leaves and after processing, thylakoid membrane fragments (0.5 - 2.8  $\mu\text{m}$ ) were obtained, which *showed surface active properties*; their adsorption kinetics was typical for large molecules or soft particles. The thylakoid fragments adsorbed at the oil/water interface and effectively stabilised emulsion droplets, even though droplet aggregation was observed already during emulsion preparation and increased with increased thylakoid concentration. It was suggested that the droplet aggregates are formed through bridging in a similar manner as the native membranes arrange themselves in their native 3D conformation (i.e. stacking)
- *Leaf side-product (pulp)* mostly consists of *cell wall components* such as cellulose, hemicellulose, pectin, lignin and structural proteins. Aqueous purification of the leaf pulp *removed soluble material* and resulted in particles that are rich in dietary fibres (~78 wt%) and still contained some residual protein (~6 wt%). These so-called cellulosic particles showed *spontaneous adsorption to the oil-water interface* that was attributed to deformability of the particles, probably due to swelling after suspending in water. In an emulsion system, the finer cellulosic particles stabilised the emulsion droplets against coalescence, while larger particles immobilised oil droplets in a stable cream layer.
- The results of the study were considered from an *industrial perspective*. Various options for processing sugar beet leaves were evaluated on their resource use efficiency. This included stabilising routes to prevent spoilage, and decentralised processing to improve both resource use and possible effects on the land. The use of resources was assessed in terms of energy requirement and exergy indicators. A *freezing* and a *decentralised* process was evaluated. The one that was found more suitable was the *decentralised* processing, which allo-

cates the pressing of the leaves towards the farms and enables direct returning of leaf pulp to the land for nutrient recycling.

- Detailed discussion using available literature is provided for processing towards enriched fractions, routes for process optimisation, integrated process routes are discussed in detail for leaves biomass for protein extraction.

For future research on green leaves:

- The production of pure proteins is probably *not economically attractive*. However, the total use of leaves may well become attractive by producing functional fractions for high-value uses in food, and by using residual fractions for other, lower-value applications.
- The integrated approach developed on this study is expected to *maximise the valorisation of leaves* and avoid inefficient supply chains.
- Changing the approach to processing leaves also implies changing the approach on how functionality is defined: A striking example coming from this study on this argument is the *combined functionality of thylakoids*: functionality of thylakoid fragments stabilizing oil-water interfaces providing Pickering O/W emulsions in combination with the satiation effect attributed to thylakoid membranes.

#### Summary of discussion with Dr. Angelica Tamayo Tenorio

The overall aim of the thesis was to obtain understanding on the use of green leaves as a food source by exploring currently neglected leaf fractions. As green plant leaves, sugar beet leaves were used as model material. Samples were mainly obtained by harvesting in WUR facilities.

The green plant material contains thylakoid membranes, which are rich in hydrophobic compounds such as hydrophobic proteins. The sugar beet leaves were cut and then blended fresh. The slurry was then centrifuged and the pellet was analysed for hydrophobic and hydrophilic protein extraction using a huge variety of different methods.

The pellets were analysed and was found that they contain among others a lot of lipids, pigments, and proteins. Moreover, the presence of other hydrophobic compounds along with the proteins

had a big negative impact on the protein extractions and isolation. For instance, after a 2 step centrifuge procedure, it was provided an extract of approximately 45% protein content with about 20% yield.

The results of protein extraction for either hydrophilic or hydrophobic proteins showed that their availability was very low (low yields and purities). At the same time it was very difficult to isolate the different types (molecular level) of proteins due the large variation of the molecular weight found on the pellet.

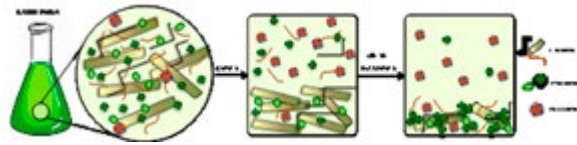
Therefore, the focus of the project was changed; the main study was focused on the functionality and applications of the protein extract (not protein itself) for food applications. In terms of functionality the extracted protein rich powder was tested and it was shown that can be used in various type of food product development applications. For instance, the most unexpected findings were that 1) the extract can be used as a stabiliser for Pickering O/W emulsions; and 2) (through literature) the extract can be used in food products in order to provide a satiety effect to the consumer.

*Industrial partners:* While in the beginning there were few industrial partners involved in the project only one remained till its end. Angelica believes that industrial partner provided high value on her project by fruitful discussions and good suggestions for her research.

### Summary of discussion/interview with Prof.Dr.Ir. Atze Jan van der Goot

Description of project and main results in short: Leaves have potential to become a source of protein and other valuable components. In the project protein fraction with a purity of 20-25% and a yield of 50% were obtained by pressing the leaves, a heating step and a centrifuge step. The extraction was done on wet leaves (separation in dry leaves is very hard). In order to test the former, sugar beet leaves were tested as a model system. The results showed that is difficult to use sugar beet leaves as potential commercially available source for protein extraction. This due to the availability of proteins.

At the end of the project, the extraction of proteins from different types of wet biomass was compared with extraction of protein from legumes. This was done based on literature data. Using different extraction methods it was shown that proteins can only be extracted in fractions with either high yield and low protein purity or low yield and high purity. By performing a detailed literature study it was shown that these results are in line with previous studies on wet plant stream protein sources, which all can fall on a same line by plotting protein purity vs yield for the extracted fractions.



Same analysis for the protein fractions extracted from oil crop/pulses-based biomass showed that they also fall on a same line providing though better protein fractions; e.g. fractions of same yield provide higher protein purity for oil crops/pulses-based biomass.

The low availability of proteins (low yield low purity fractions with respect to the legume-based biomass) from wet biomass was attributed to 1) high water content (90%) of wet biomass; 2) huge difficulties on processing the dry biomass; and 3) the microstructure characteristics of the wet biomass with respect to protein crops. In protein crops, proteins are present on micron size structures while on leaves and wet biomass, proteins are present on structures which are typical in the range of few nm.

Potential applications:

- For food application, extraction of protein fractions from leaf biomass may have some applications as high value component. Besides the issues described above concerning the availability of proteins of wet biomass, the project leader's opinion is that for food application in general we should not aim for high purity fractions. In the human diet, we don't eat protein isolates but food containing proteins along with other components.
- In bulk applications, the aim could be for feed, after applying purification step(s) of the bio-

mass. One drawback is considered to be the high water content. Besides the processing challenges, high water content of biomass also provides practical obstacles for real applications such as difficulties on the logistics.

- Biobased applications are not considered as an option since the bottle neck is the high water content.

*Industrial partners:* The project leader finds the contribution of industrial partners of high value in the project.

For further investigation, and to significantly improve the protein purity and protein yields, it is important to gain more knowledge concerning the extraction from green fresh biomass in relation to

the extraction from legumes. One aspect to involve is the aspect of the biological function of the different proteins in the biomass.

Further, more knowledge is needed on the type of protein, and the requirements in different end applications. The bulk application for proteins is the use in feed.

In addition, the logistics concerning wet protein based biomass has to be investigated. Especially, in November, December and January a number of wet products are available. How to deal with wet streams is an important aspect to be addressed in the future.

## ALGAEPRO4YOU: Extraction and fractionation of functional proteins from microalgae for potential application in the feed or food industry

PhD candidate Edgar Suarez Garcia, Project leader: Prof.Dr.Ing. Michel H.M. Eppink

### Summary of discussion with Edgar Suarez Garcia

From the algae strain of *Tetraseimis suecica* an extraction process is applied which consists of bead milling and an aqueous two phase extraction through centrifugation. The functional fractions: pellet and top phase are then investigated for their functionality for food applications.

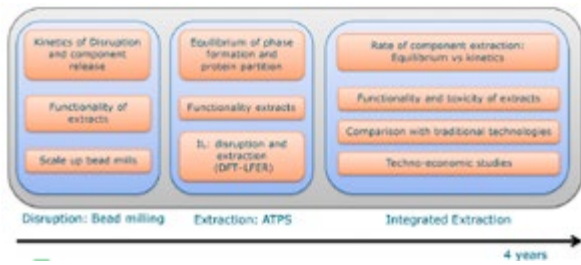
The focus is not on trying to provide pure protein product but to investigate the non purified extract for potential food applications.

The project structure is as follows:

- In the first step of the process (bead milling) the areas of focus is on: 1) the kinetics of disruption and component release from algae; its is investigated using green solvents such as water and ionic liquids, 2) the functionality of extracts; without further purification the extract is not pure in protein but has good functionality which is tested for their: gel, foaming, emulsion and solubility ability. The state of the project is currently focusing on efforts to scale up the bead milling. Practically: The extraction process is the same with the one that was used in the

past for protein extraction from another algae strain. The results showed that the process is also applicable for algae strain of *T. suecica* which is tested on this study.

- On the the second step of the project the aim is to investigate the extraction process. For aqueous two-phase systems (ATPS) for protein separation, the focus is on 1) the equilibrium of phase formation and the protein partition and 2) providing modelling tools (DFT-LFER) combing the disruption step of the bead milling with the extraction step through ATPS.
- The final part of the project is to investigate the rate of component extraction in order to find the links between equilibrium and kinetics processes, the functionality and the toxicity of the extracts. The last part is dedicated to compare the applied extraction methods on available traditional technologies and to perform techno-economic studies for the valorization of the results.



**Industrial partners:** only one industrial partner is still actively involved in the project and Edgar believes that its contribution is of high value. The industrial partner contributes to discussions and have suggestions to the content of his project and provide guideliness for his future research.

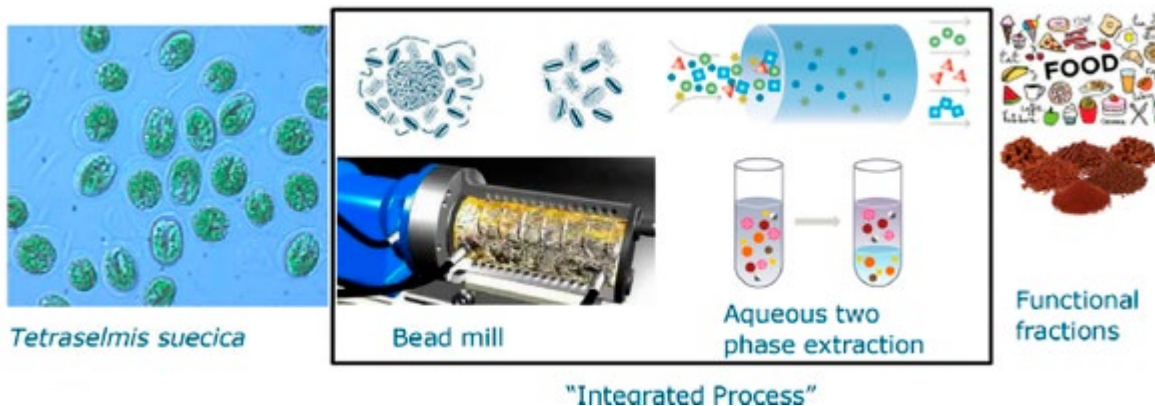
**Summary of discussion with Prof. Dr. Ing. Michel H.M. Eppink**

The aim of the project is the extraction and fractionation of functional proteins from microalgae for potential application in the feed or food industry. The algae strain *T. suecica* was used as source of biomass. At BPE the PhD candidate is focussing his research on the biomass, the cell disruption and the extraction of the protein. In detail, wet bead milling process has been applied in order to disrupt the algae and release the functional components. To decrease the energy input, short bead milling times were applied using beads with different sizes. In the end, 50% of the proteins can be extracted with a purity of 65%. After bead milling an aqueous two phase extraction process was applied to obtain protein rich fractions. Surprisingly the results showed that by lowering the energy it was possible to obtain protein fractions of similar protein content. This was achieved by reducing the size of beads. The approach was tested for other type of algae strains and the results showed similar or even

improved behaviour. The kinetics of disintegration and component release was improved for two other algae strains at lower bead sizes with much lower energy consumption. The native structure of the released was retained for all strains during the process. The techno functional characteristics of the protein rich extracts from this method are evaluated by another PhD student in FBR. The results so far are showing functionality similar with the functionality of extracts obtained from the traditional method.

At this point the efforts are concentrated on scaling up the described processing method, by using theoretical calculations. The aim is to provide rules on scaling up the process. The project leader is very optimistic that this will be accomplished much even sooner than expected. The next step of the project is to run pilot plan experiments to validate the results.

For next steps in the future, from industrial point of view there is no interest on shifting the research towards developing techniques or processing procedures aiming for high purity/yield fractions. It is considered as too expensive (money, energy, input) to achieve fractions with improved protein purity/yield fractions and therefore of no industrial interest. In principle algae strains are considered a sustainable source of proteins by the project leader, as long as high protein extracts can be used for applications (food or feed) with as minimum as possible processing steps. This is indeed the aim of the current research. The highlights of the PhD research so far show a focus on decreasing the input energy and optimizing the process to a more sustainable one. According to the project leader, this research fits very nicely in





the area of using more plant proteins for the future. In order to achieve that, another issue to be addressed is the improvement of the properties (functional, taste etc) of these protein extracts. The way to work is towards finding solutions on the techno functional issues of plant proteins. The

project leader believes that is key that more food companies with more commitment should be involved in the research. This is necessary to test the different protein samples in end applications and to look for their potential.

## STW- Progress - Proteins from green sources for use in both food and fish feed

*PhD candidate: Emma Teuling, Project leader: Dr. Ir. Peter A. Wierenga, Summary of discussions with Dr. Ir. Peter Wierenga*

The aim of the project is to obtain protein rich fractions from green sources. The approach in the beginning of the project included the evaluation of different green sources of biomass for protein extraction. Under the scope of investigation was: Extracting proteins from beet leaves, extracting proteins from grass, proteins for fish feed and proteins from algae. Beet leaves as a source of green biomass was a topic that was addressed in another STW project and therefore was abandoned. Attempts for collaboration of the two groups and combination of the research turn out fruitless. Concerning protein extraction from grass, complications with polyphenolic compounds were identified and a technical issue; no availability for pilot plant trial, shifted the research to other type of green sources of biomass. Research of protein for fish feed was an option but became a less interesting topic after the company involved stepped away from wet processing methods. Therefore, the PhD research focussed on the extraction of protein from different algae strings.

Four different algae strains were tested namely *Chlorella*, *Arthrospira platensis (spirulina)*, *Nannochloropsis*, and *Scenedesmus.sp.* For the extraction of proteins the method used, is similar to the one developed in the past by Anja Schwenzfeier. The method was developed for algae strain *Tetraselmis sp.* and involves bead milling and centrifugation steps separating water soluble from insoluble components (technology developed in P. Wierenga group). There was no interest on trying to modify/optimize the extraction method neither further fractionation was applied for obtaining high

purity protein fractions. By applying the same extraction conditions on the four different algae strains an interesting finding was emerged. The cells of the four algae strains are different and this has an impact on the protein extraction efficiency. This is attributed to the hardness of the cell wall and a correlation could be built among the parameters of the bead milling step: time and flow rate with the release and therefore with the protein extraction. On algae, proteins are found binded in the cell wall material along with other components (e.g. cellulose). During the bead milling process the cell wall is disrupted releasing the proteins. The hardness of the cell wall material of the four algae strains is different and as a result this has an impact on the protein release and therefore on the protein extraction efficiency. On protein extraction results obtained from the above described processes had yield / purity (7-10% yield, 60-70% purity). These results are in line with values obtained from previous studies of project leader's group.

The next steps of the study were dedicated on the characterization and the techno functional properties of protein rich fractions, which is the main focus of the PhD research. For the characterization of the proteins, different methods were applied including the determination of amino acid composition and protein content, spectrophotometric analysis and determination of the molecular weight (SDS-PAGE). The techno functional properties of proteins and protein rich fractions were evaluated. Protein rich fractions were investigated for their water solubility, foaming and

emulsifying properties (article prepared) as function of different parameters (e.g. pH and temperature). The results obtained concerning the techno functional properties of the protein extracts are in line with previous studies of projects leader's group. Most interesting results obtained for proteins extracted from one type of algae strain; which showed to have high solubility (with respect to plant and common algae proteins) in broad range of pH values. This highlight of the PhD research could have a big impact for food product development applications aiming for products like athletic drinks or high protein bread. From an application perspective this finding is of high value since algae proteins do not contain polyphenol oxidase which is a clear advantage in comparison with common plant proteins like pea and sunflower.

For pragmatic applications of the PhD project, fish feed (in vivo) trials were performed testing the algae strain *Nannochloropsis* with and without treatment of bead milling.

As steps further the interest of the project leader, is to focus on the techno functional properties of protein rich fractions from sustainable sources in general. In relation to algae proteins he believes that the focus should be applied on 1) research on the insoluble protein fraction, 2) comparison with legume proteins, and 3) colour removal. Finally, information is needed on amounts of protein concentrates and isolates in food and feed. In addition, information is lacking on which functionality is exactly needed for which application.

## Production and valorisation of high quality proteins from insects (In2Food)

PhD (Final stage) Renske (H) Janssen, Project leader: Dr. Ir. Catriona MM Lakemond

### Summary of the work

The In2Food project is interdisciplinary and addresses several research areas: (1) extraction and characterization of soluble proteins in relation to enzymatic browning (Renske Janssen WUR FQD/FCH), (2) usage of whole insect (FBR); (3) large-scale automated rearing of insects (WUR-Entomology); (4) providing food and feed safety data during insect protein production (RIKILT). The overall objective of the proposal was to provide a knowledge base for the use of insect protein in food and feed. The project's key-focus was on the lesser mealworm (*Alphitobius diaperinus*), also yellow Mealworm (*Tenebrio Molitor*) and BSF (*Black Soldier Fly*) were studied.

The PhD results showed that the protein concentration of the supernatant is relatively high reaching to weight fractions up to 70-80% protein. Protein extraction possibilities with none or limited enzymatic browning were developed and insight in browning mechanism and specific enzymes responsible was obtained. Specifically, insect specific nitrogen-protein conversion factors were determined. Currently, the techno-functional properties of lesser mealworm proteins, such as

digestibility and gelation, as a function of type of processing and enzymatic browning are being investigated.

At WFBR focus was on the use of whole mealworms. The influence of (pre)processing of whole mealworms on formation of brown colour and texture was studied. Also a short study on where to apply mealworm paste in products was performed by performing a database study on current (new) food applications for mealworms.

At RIKILT and WU-ENT, they performed a study to investigate the potential accumulation of cadmium, lead and arsenic in larvae of two insect species, being *Tenebrio molitor* (yellow mealworm) and *Hermetia illucens* (black soldier fly). An experiment was held with 14 treatments, each in triplicate, per insect species. Twelve treatments used feed that was spiked with cadmium, lead or arsenic at 0.5, 1 and 2 times the respective maximum allowable levels (ML) in complete feed, as established by the European Commission (EC). Two of the 14 treatments consisted of controls, using non-spiked feed. Development time, survival rates and fresh weights were similar over all treatments, except for

development time and total live weight of the half of the maximum limit treatment for cadmium of the black soldier fly.

Bioaccumulation (bioaccumulation factor > 1) was seen in all treatments (including two controls) for lead and cadmium in black soldier fly larvae, and for the three arsenic treatments in the yellow mealworm larvae. In the three cadmium treatments, concentrations of cadmium in black soldier fly larvae are higher than the current EC maximum limit for feed materials. The same was seen for the 1.0 and 2.0 ML treatments of arsenic in the yellow mealworm larvae. So, if insects are used as feed materials, the concentration of these elements in the substrate should be considered carefully.

Suitable substrates are needed to efficiently produce high quality insects. Organic wet fraction (ONF) and 'digestaat' were provided by one of the industrial partners and tested in black soldier flies (BSF). This species had a low survival and developed poorly on these substrates. Combinations of DDGS and spent grains were tested in BSF and in lesser mealworms. BSF developed well but had a lower final weight than on their control feed. Lesser mealworms developed slower and had a decreased survival on the aforementioned mixtures. These two species, as well as house crickets were provided with diets enriched with omega 3 fatty acids. In all three species this strongly decreased the omega 6/omega 3 ratio, which is considered beneficial for health. Lastly, substrates which were contaminated with aflatoxins were provided to BSF and yellow mealworms. Both species developed well on the contaminated substrates and did not accumulate aflatoxin. This indicates that they can be used to upcycle feed that is unsuitable for conventional production animals.

One of the main bottlenecks of BSF production in temperate zones is the induction of mating and oviposition under artificial light conditions. After determining the spectral sensitivity of photoreceptors in the compound eye of this species we provided adult flies with light of wavelengths for which the photoreceptors are maximally sensitive. This strongly increased the number of produced larvae.

BSF larvae are suggested as a suitable feed ingredient for several production animals, including broilers. Although broiler growth was lower when higher concentrations of live BSF larvae were provided, broilers used their feed more efficiently, and had improved welfare compared to broilers provided with conventional feed.

Several smaller research questions that came up during the project were picked up and tackled by the research team. The direct involvement of companies from several parts of the insect production chain resulted in direct applications of the knowledge developed during the project. Furthermore, the results on safety are very relevant with relation to building a novel food dossier.

#### Summary of discussions with Renske (H) Janssen

The aim of the In2Food project is the production and valorisation of high quality proteins from insects. Enzymatic browning is considered as one of the main drawback for the protein rich extracts obtained from insects and their use in product development for food applications. This is exactly the area of research of the PhD project. The project is dedicated on the study of the effect of enzymatic browning during the extraction on proteins from insects. To investigate that, different types of insects (i.e. yellow mealworms, lesser mealworm and black soldier fly) are being studied for obtaining protein rich fractions. Depending on the species, insects are either provided by an industrial partner or from entomology department at WUR. All insects are processed by the PhD candidate: The whole insects are blended and then the slurry is used for analysis and in order to study the enzymatic browning.

Most of the times aqueous suspensions are used for centrifugation, while the pellet and supernatant obtained after the centrifugation step are used to study the enzymatic browning. The protein concentration of the supernatant is relatively high reaching to weight fractions up to 70-80% protein. Different parameters are varied in order to test the effect of enzymatic browning; these include temperature, pH, processing steps (e.g. freeze drying, heating), time of storage etc. For instance, increase of pH can increase the purity and yield of the protein fraction, but the extracted proteins are



sensitive to high temperature (> 60oC) and they get denatured.

Finally, for the involvement of industrial partners in the project, the so far collaboration is good and the PhD candidate believes that the companies involved, provide good input on her project by not only providing materials but also have valuable input and suggestions helping for her research. There is also active participation and collaboration of the PhD candidate along with the other partners involved in the project through the STW meetings.

#### Summary of discussion with Project leader:

Dr. Ir. Catriona MM Lakemond

In the beginning of the discussion the project leader provided the general scope of her research: The study of insects as a potential source of valuable components for food applications.

Insects are already consumed in many parts of the globe and are a sustainable protein source. However, the use of insects for food and feed applications in Europe is in its infancy, although the insect industry is developing rapidly at the moment. Insects offer a high quality, efficient and sustainable protein alternative, considering feed conversion rates, greenhouse gas and ammonia emissions, and the capacity to valorize organic side streams. As such, insects can close the loop in a circular economy (ECO:nomics, creating environmental capital). Insects can be grown on waste streams arising from the food and agricultural industry, converting these into high quality protein products. Recognisable insects are not accepted in the Western world as a food source for humans for cultural reasons. Processing insects and extracting proteins are ways to overcome this consumer reluctance and this needs to be developed. Insect extracts will provide a higher added value than the side streams or the insects themselves. During extraction enzymatic browning due to polyphenols can occur. The browning reaction impairs the visual attractiveness of insect-derived products, as well as their functionality

Innovative and sustainable insect production can contribute to global food security, either as feed or directly as food for humans. Nutritionally, insects are comparable to conventional meat such as

poultry, pork, beef, or fish. Insects contain 30-70% protein on a dry matter basis.

Given the fact that insects as a food or feed component is relatively new, several disciplines should work together in order to have in the future, insects or insect components in food applications. Working towards that, the project leader's research is interdisciplinary and requires the collaboration across several disciplines and departments. In line with the developments in the insect sector 1) rearing, 2) harvesting, 3) extraction of relevant food components, 4) safety (novel food), 5) food and feed applications, 6) usage of side-streams to feed insects and 7) consumer acceptance are areas of interest require collaboration to provide a close loop for real applications of food components extracted from insects.

Concerning future aspects:

As steps to the future, the Project leader finds most important the continuous collaboration of industry with the multidisciplinary conducted research. Research on consumer acceptance, health risks, valorisation, rearing, food applications should strongly be integrated in order to move forward in knowledge development. So collaboration along the entire insect value chain is necessary.

Further research:

The project leader believes that more work is necessary on protein extraction and identification in relation to functionality. This within the framework of insect rearing, especially with relation of the composition of the gut on the moment of harvesting. Less purified protein fractions obtained during the protein extraction are more sustainable and less costly. The focus of the research could be the functionality of these fractions in relation to food or feed applications. Also the effect of heating on the properties of proteins, like digestibility is an interesting area of further research. For future building of novel food dossiers further safety aspects of insects fractions should be studied.

Conference:

Also a conference/workshop on insects 'INSECTS; THE VALUE CHAIN' will be held on March 21 and 22 2018 to disseminate the results obtained in the In2Food project and to build further relations with the industrial partners.

## Colophon

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