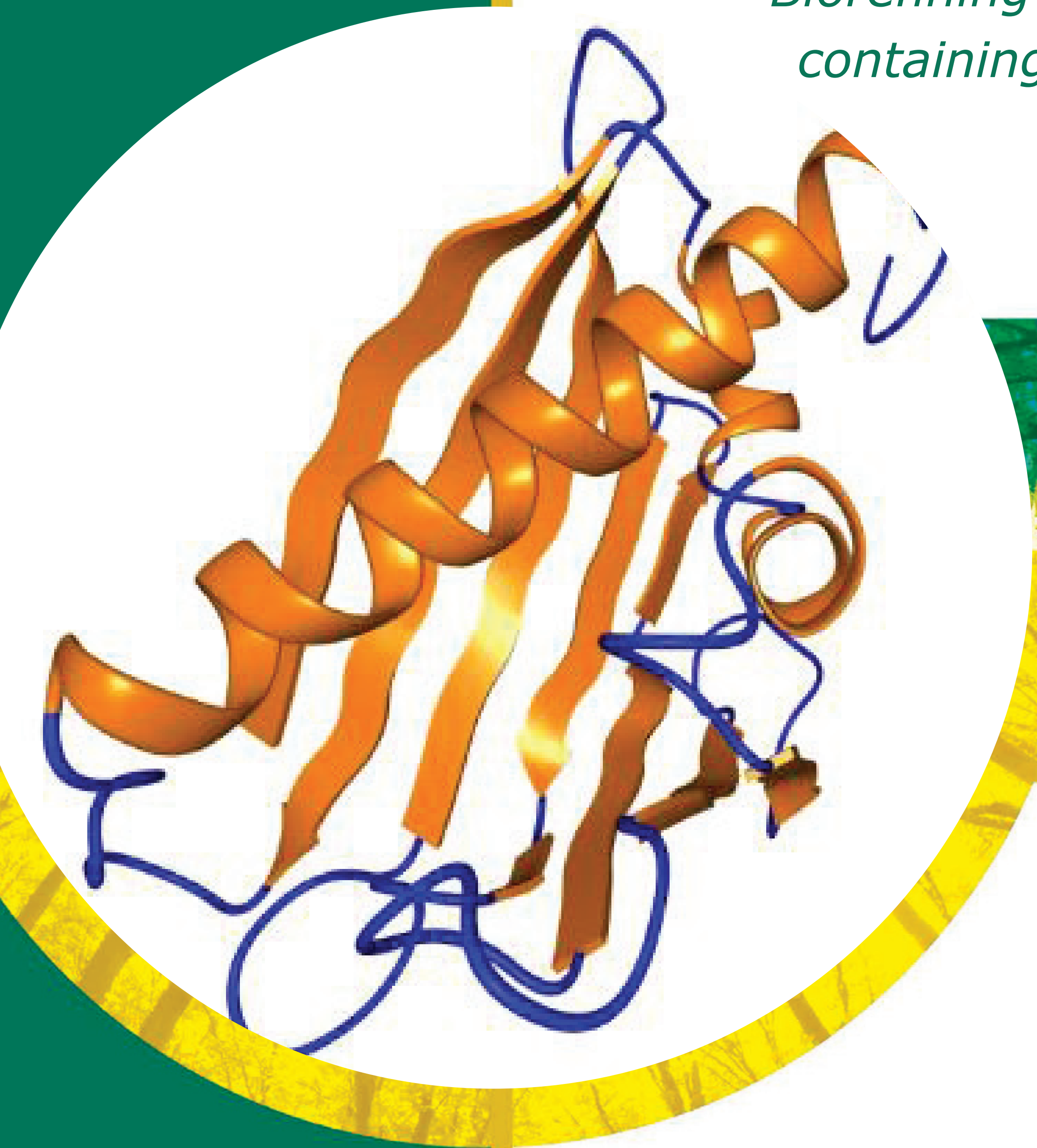
Proteins for Food, Feed and Biobased Applications

Biorefining of protein containing biomass



This publication provides an overview of protein containing biomass sources & refining technologies; and protein types, markets & market trends. It is prepared to inform non-protein experts, for example stakeholders from the energy sector, to show the market opportunities for the sustainable valorisation of biomass-based protein fractions to increase the overall market competitiveness of full sustainable biomass value chains in a Circular Economy.

IEA Bioenergy

IEA Bioenergy: Task 42: September 2016

Proteins for Food, Feed and Biobased Applications

Biorefining of protein containing biomass

Report prepared by

Wim Mulder, Carola van der Peet-Schwering, Nam-Phuong Hua, René van Ree (Wageningen University & Research) with input from the NTLs of IEA Bioenergy Task 42

ISBN: 978-1-910154-29-8

Published by IEA Bioenergy Task42

1. Introduction

Background

Within the Circular BioEconomy sustainably produced biomass will be 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.

Various biomass resources will be fractionated into their composing biobased intermediates (such as proteins, sugars, oils and fibres/lignins) that will be further processed by biochemical and/or thermo-chemical (catalytically supported) pathways to marketable biobased products and bioenergy.

Biomass resources rich in proteins (> 10 wt%) are currently mainly used in the food and feed sectors, however, their use is mostly very inefficient. By the separation of protein fractions from these resources and upgrading to specific food and feed ingredients, using the residues for valorisation to other biobased outlets, the biomass resources can be used more sustainably.

Depending on the type of crops and proteins, separation and further valorisation to non-food/feed technical applications is another interesting potential valorisation strategy.

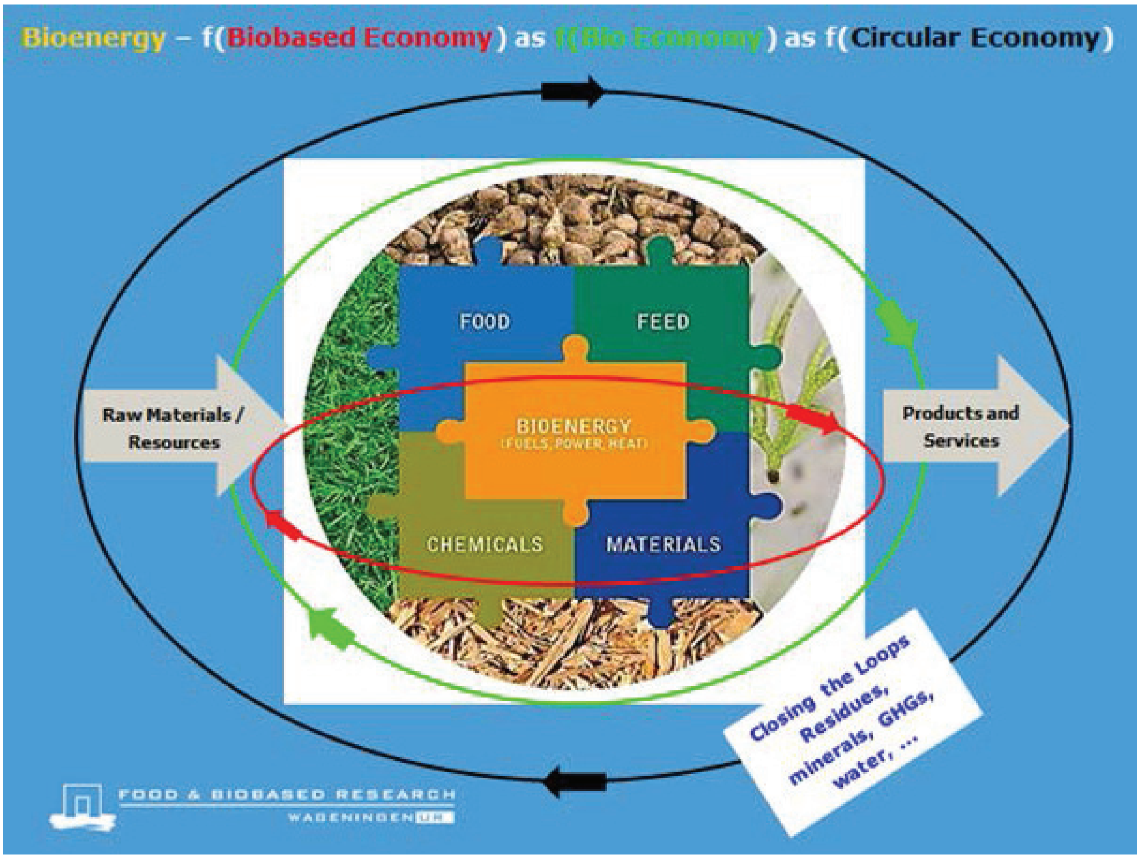


Figure 1. *Schematic representation Circular BioEconomy (IEA Bioenergy Task42, 2015)*

Problem definition

Within the Circular BioEconomy stakeholders from different market sectors have to work closely together for joint successful sustainable valorisation of the available biomass resources potential. Efficient use of available biomass resources using bio-cascading and biorefining approaches producing a spectrum of marketable biobased products and bioenergy is needed to be able to deploy full biomass production – conversion – end-use chains that are market competitive with their fossil and alternative renewable primary resources.

The knowledge specifically on proteins, and on their valorisation opportunities and potential market outlets, is lacking to a large part of the stakeholders active in the Circular BioEconomy, specifically to those not active in the food and feed industry. This is a problem, because it is important to be aware to a certain extent on the full market valorisation potential of all constituents of the biomass resources to be able to jointly develop the most promising joint valorisation strategies.

Objective

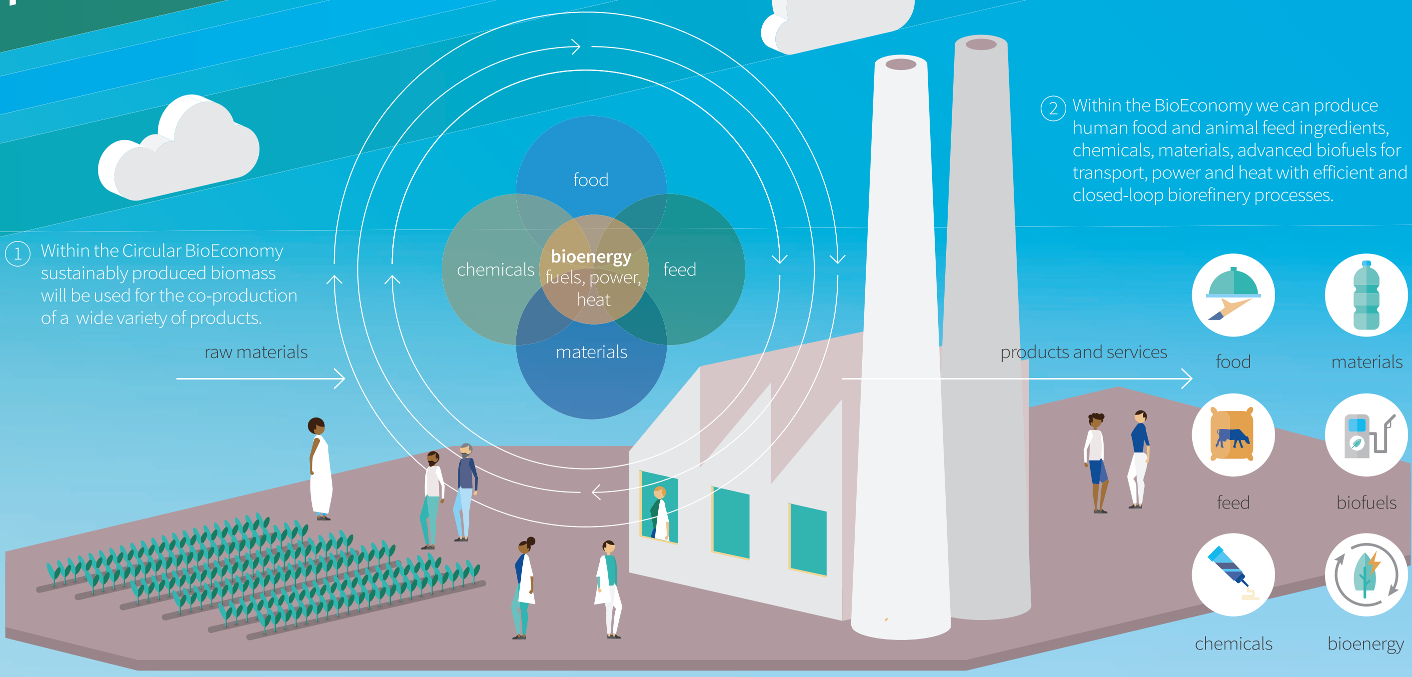
The aim of this report is to provide an overview of protein containing crops, types of proteins and side products and their current applications, and market potentials for food, feed and biobased applications. This info can be used by all stakeholders active in the BioEconomy to jointly develop integral sustainable biomass valorisation strategies. Specifically for the energy sector, this report shows that upstream protein extraction prior to the conversion of biomass into "energy" and/or co-valorisation of protein-rich agro or process residues will add economic value to the overall valorisation of available biomass resources, thereby improving their overall commercial deployment potential.

Report set-up

Types, amounts and prices of protein-rich biomass sources that are being cultivated are addressed (chapter 2). The focus of this report is on plant proteins because for the energy sector mainly side streams from the agro-industry are relevant. The report describes the type of proteins that traditionally are being used in food and feed, and the requirements for their use (chapters 3 and 4). Worldwide there is an increasing demand for proteins, therefore, also novel protein sources for food and feed that are currently under investigation are being discussed. In comparison with feed and food, a minor amount of proteins is being used in technical biobased applications, such as adhesives, coatings and chemicals (chapter 5). These applications are being discussed, and also the perspectives of the use of the proteins in the future. Concepts being used or developed for the biorefining of protein containing biomass sources are described in more detail (chapter 6).

At the end of all chapters, the main messages are summarised by means of an infographic. These infographics can be used by readers that want to have a quick and short overview of the major opportunities of protein valorisation within the Circular Economy without the need to read the full report.

Proteins in the BioEconomy



(1) Within the Circular BioEconomy sustainably produced biomass will be used for the co-production of a wide variety of products.

(2) Within the BioEconomy we can produce human food and animal feed ingredients, chemicals, materials, advanced biofuels for transport, power and heat with efficient and closed-loop biorefinery processes.

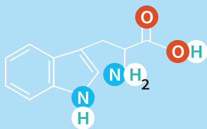
Amino acids

All proteins are made up of a combination of 20 alpha amino acids.

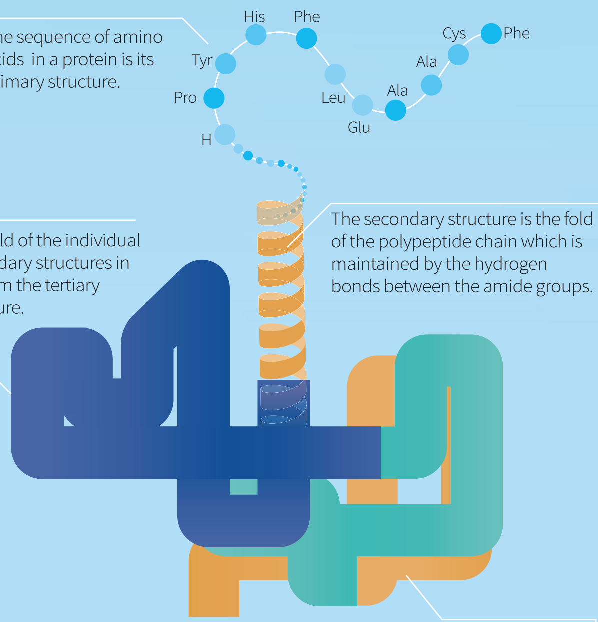
Glycine is the smallest amino acid

- Gly
- Ala
- Val
- Leu
- Ile
- Pro
- Phe
- Tyr
- Arg
- Ser
- Thr
- Cys
- Met
- Asn
- Trp
- Lys
- Gln
- His
- Asp
- Glu

A more complex amino acid is tryptophan



Protein structure



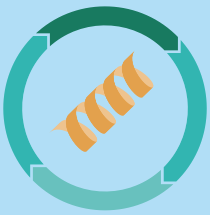
The sequence of amino acids in a protein is its primary structure.

The secondary structure is the fold of the polypeptide chain which is maintained by the hydrogen bonds between the amide groups.

The fold of the individual secondary structures in 3D form the tertiary structure.

The quarternary structure is the 3D-structure of several polypeptide chains and non-proteinaceous parts.

Protein application



Proteins can be used in a wide range of applications in the BIoEconomy, from ingredients for human food and animal feed to resources for industrial applications (chemicals, materials).

The unique properties of proteins and the multitide of applications place them at the heart of the BioEconomy

2. Protein containing sources

Proteins can be found in several agro-materials, plants and animals. Proteins play an important role in the diets of animals and humans because of their nutritional value (essential amino acids). Most of the protein uptake by human beings and animals is by eating meat, fish, protein-containing crops, such as legumes, and dairy products, such as milk. This chapter focusses mainly on plant-based proteins because they potentially can add value to bioenergy-based value chains if they can be extracted,, functionalised and co-valorised to biobased products for food, feed and industrial applications.

Traditionally, for food consumption cereals (e.g. wheat, barley and sorghum) and legumes (green peas, lentils, beans and chick peas) are being grown. The production volume of protein crops, on both European level and on world level, is being depicted in Figure 2. 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. Together with rice, wheat, potato and soy bean these products cover more than 80% of the production of protein crops worldwide. These crops, however, differ in protein content and dry matter content.

By far, the largest amount of proteins is being used in feed. Relatively small amounts of isolated proteins as concentrates (protein content higher than 65%) or isolated (protein content higher than 80%) are being used in food and feed (see table 7). In addition, even smaller amounts of proteins are being used in technical applications, such as adhesives (see table 16).

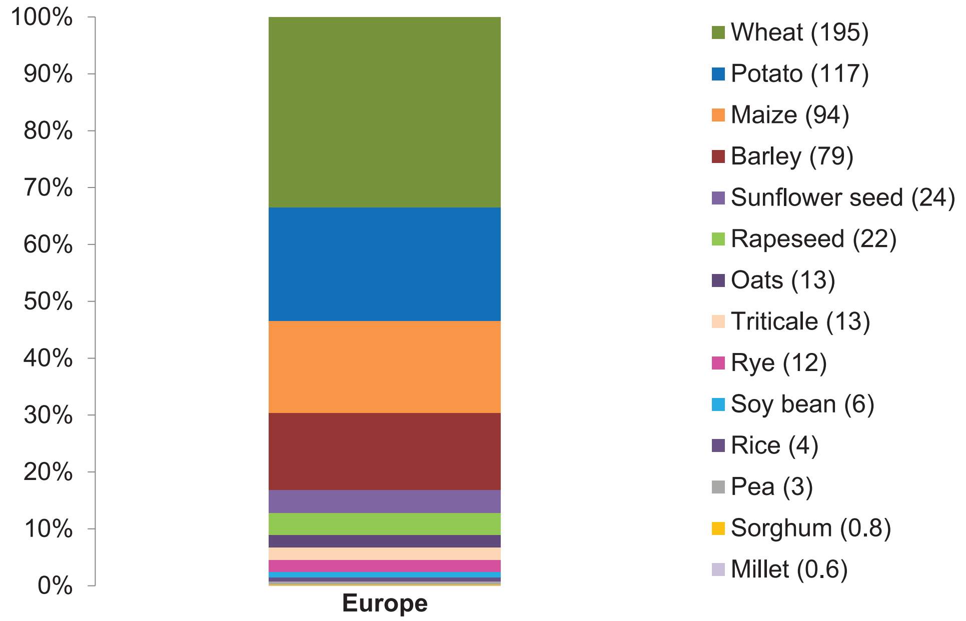
Protein amounts in feed (EU): about 60-70 megaton

Protein amounts in food (EU): about 20 megaton

Protein concentrates and isolates amounts in food (worldwide): about 1500 kiloton

Protein amounts in biobased applications (EU): about 200 kiloton

In recent years, due to the increase of the world population, a durable protein supply for humans and animals for the future is placed on the political agenda. To avoid protein shortages in the future, protein losses in the whole chain should be minimised. On the other hand several protein sources have not been used optimally. Biorefinery research programmes are being carried out focussing on the isolation of valuable compounds, like proteins, from various crops, and agro and process residue streams. As an example, the recovery oil from oilseeds like sunflower seeds or rapeseeds leaves behind press cakes with a higher protein content. Also new protein containing biomass sources, such as algae and grass, have become the subject of studies.



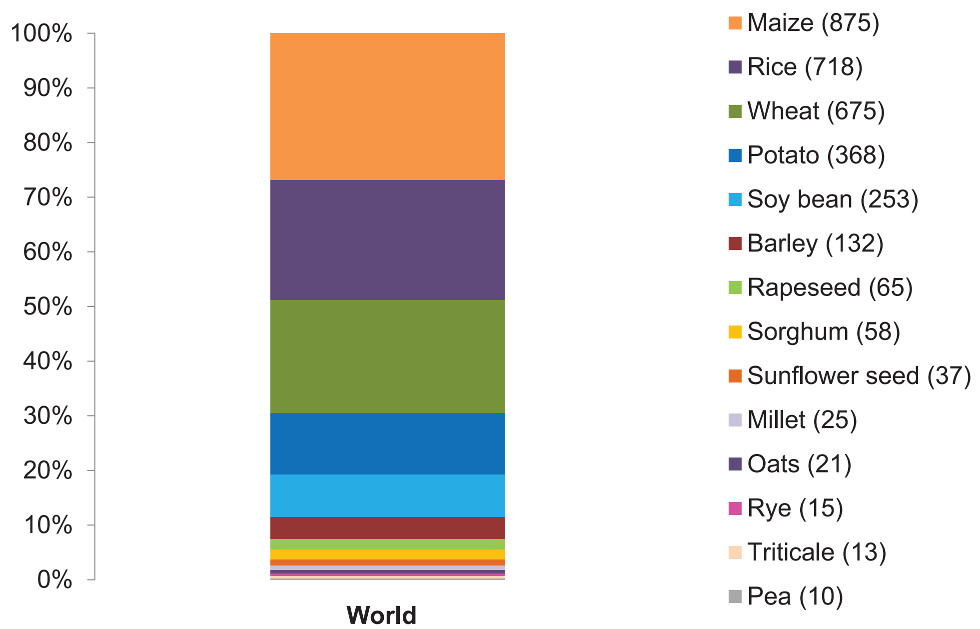


Figure 2. *Production (million tons) of protein containing crops*

In Table 1, the prices for a few of these protein crops is depicted, generally ranging between 0.10 and 0.50 €/kg.

Table 1. *Prices of protein crops for food (2011)*

Protein product	Price [€/kg]
Soy bean	0.41
Rapeseed	0.30
Maize	0.12
Sunflower seed	0.33
Bread wheat	0.10
Durum wheat	0.14
Barley	0.09
Lucerne	0.12

Apart from protein-crops that are grown for food consumption, there are a variety of residue streams from the agro industry containing proteins. Biomass can be classified according to its protein content (Table 2). The group with a high protein content, like soybean meal, has many possible applications with a high value, usually as animal feed. The second group with a protein content of about 25-45 wt% has lower economic value but is suitable as feed for pigs. The economic value depends on the protein content, but also on the presence of starch/sugars. Besides this, the presence of anti-nutritional components (for example in rapeseed products) can play a role in the determination of the price. Also, the availability of the products (good versus bad harvest) has an effect on pricing. The third group has a protein content of 10-20 wt% and can be fed to cows. Usually, however, this type of biomass does not have a high value, and therefore is left on the field. The fourth group, having a protein content of 5-10 wt%, is as such not good enough to be used as feed. The same counts for the fifth group with a protein content lower than 5 wt%. In general, going from group 1 to group 5, the amount of cellulose and hemicellulose increases. This explains for example the big interest in straw as a starting material for the production of second generation ethanol. Group 3 and 4 are less interesting for second generation ethanol due to the low cellulose content, but interesting for biorefinery processes and for example isolating the proteins.

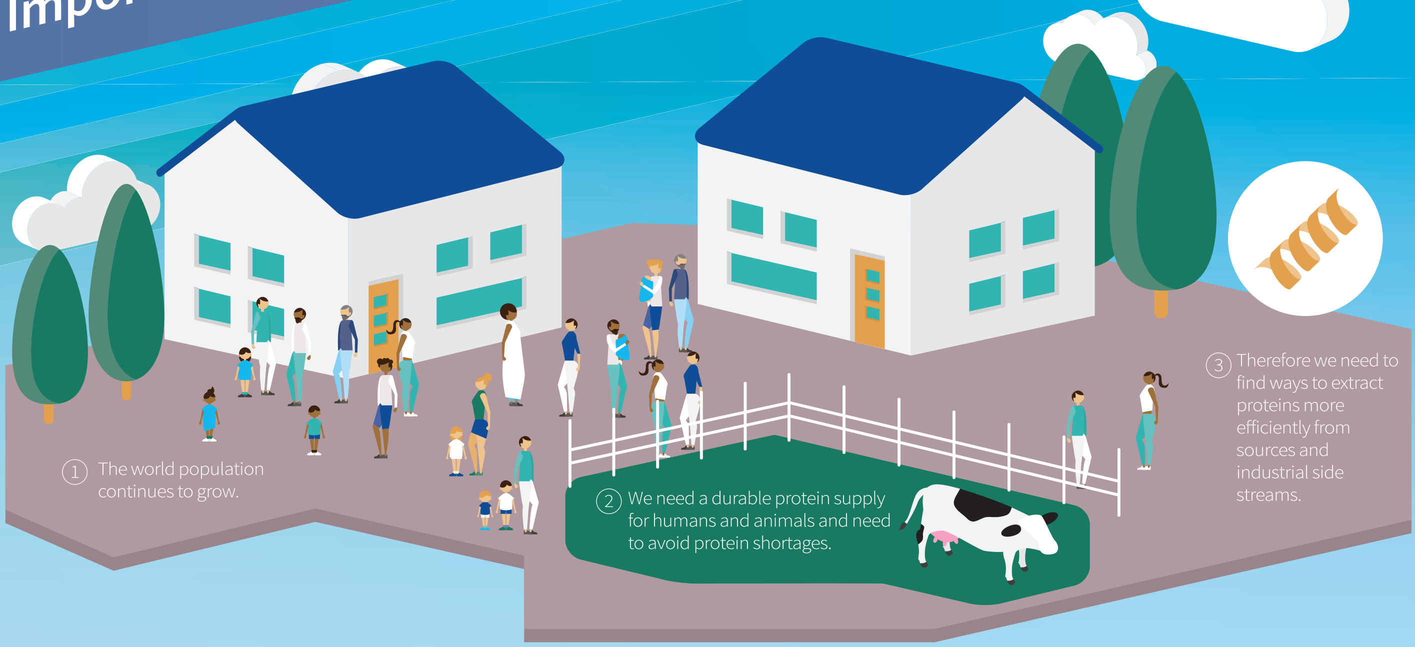
The scope of this report is to show the advantages of the use of protein-containing biomass in energy-based biorefineries sustainably and synergistically co-valorising these sources to added-value protein-based products and secondary energy carriers. Based on market volumes linking the animal feed market and energy market by means of innovative biorefinery approaches seems to be very attractive from a raw material use efficiency and overall sustainability point-of-view.

Table 2. *Protein sources classified according their protein content*

	Examples	**State of the art**
Group 1 protein content >50 wt%	soybean meal rapeseed concentrate meat meal	already applied due to high protein value
Group 2 protein content 25-45 wt%	press cake rapeseed sunflower seed meal slaughterhouse waste DDGS Microalgae	already applied (middle value, pigs)
Group 3 protein content 10-20 wt%	rapeseed hulls soy bean pods beet leaves fresh grass	applied but low value
Group 4 protein content 5-10 wt%	rape straw soy straw corn stover sunflower seed hulls	primary residue
Group 5 protein content < 5 wt%	wheat straw	primary residue

When technologies are developed that both can isolate (hemi)cellulose and protein, new protein containing biomass sources will become available that are relatively cheap. Increasing the protein content of biomass sources by biorefinery technologies will further increase their economic value.

Important sources for plant-based proteins

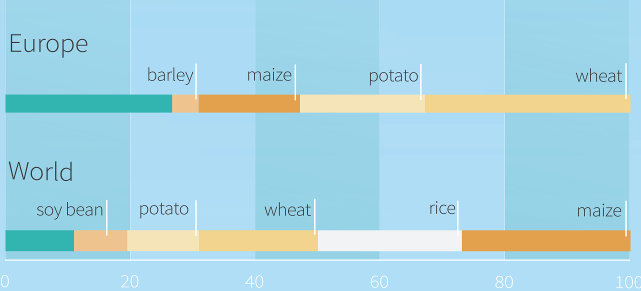


Protein uses

Proteins are mostly used in feed, hardly in industrial or biobased applications.

Protein sources

Maize is the largest crop worldwide.



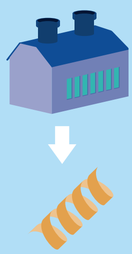
Protein content

Biomass can be classified according to its protein content into 5 groups. The top groups contain a lot of cellulose and hemicellulose, and therefore are generally interesting for biorefinery processes. Group 2 and 3 biomass sources are interesting ones for protein-based biorefinery approaches (1.: already high market value as such; 4/5: refinery costs probably will not be compensated by added-value proteins valorisation).

Protein content (%)	Group			Cellulose and hemicellulose content
HIGH	Group 1	Already applied due to high protein value	soybean meal, rapeseed concentrate, meat meal	HIGH
50	Group 2	Already applied (middle value, pigs)	press cake rapeseed, sunflower seed meal, slaughterhouse waste DDGS, micro algae	
25	Group 3	Applied, but low value	rapeseed hulls, soy bean pods beet leaves, fresh grass	
10 / 5	Group 4	Primary residu	rape straw, soy straw, corn stover	
LOW	Group 5	Primary residu	wheat straw	LOW

Innovation

Innovative biorefinery research programmes are inventing new ways to extract proteins from protein-rich biomass sources.



New sources

New biomass sources such as algae and grass are investigated.

When technologies are developed that isolate both (hemi)cellulose and protein, new proteins will become available that are relatively cheap

3. Proteins for food

Human uptake of proteins can be accomplished by consuming animal based products or plant crops. Animal protein sources include: muscle, blood, connective tissues, milk and eggs. For human consumption, important plant protein sources are cereal grains and legumes (including oil-seed legumes). The protein contents of some of the most important plant protein sources is presented in Table 3. Another source of plant proteins are nuts. Nuts are energy-dense foods that are not only particularly rich in fat but also contain a substantial amount of plant protein (source of tryptophan, arginine, lysine) (Peter Pribis, 2014).

Table 3. *Typical protein contents of major cereals, legumes, oil-seeds and vegetable sources (Day, 2013)*

Protein source	Protein content [wt%]	Other constituents [wt%]
Rice	7–9	90% starch
Wheat (flour)	8–15	~75% starch; 1–2% lipids~5% non-starch polysaccharides
Barley (de-hulled)	8–15	60–64% starch; 2–3% lipids; 3–10% soluble dietary fibre (in which 4–6% β-glucan) and 11–14% insoluble dietary fibre
Maize (corn)	9–12	70–75% starch; 3–18% oil (from the germ)
Sorghum	9–17	~2% lipids; 70–75% starch
Canola	17–26	40% oil; 12–30% non-starch polysaccharides
Chickpea	20–25	~60% starch; ~10% non-starch polysaccharides
Pea	20–30	60–65% starch; ~5% non-starch polysaccharides
Soybean	35–40	~20% oil; ~30% non-starch polysaccharides
Lupine	35–40	~10% oil; 35–40% non-starch polysaccharides

SOURCES PLANT-BASED PROTEINS

Traditionally, plant-based proteins can be grouped into 4 main sources, oilseed proteins, cereal proteins, legumes, and leaf proteins. Recently, also new sources are exploited as protein sources

Oilseed proteins

Main sources include: oilseed crops, soybeans, rapeseed/canola, cottonseed, peanut/groundnut, and sunflower seeds. Minor sources include: sesame, flaxseed and linseed. Traditionally the primary product from these oil seeds is oil where a meal provided after oil recovery is used for feed. Currently, as a plant source protein, soy proteins rank number one in food applications, where their functional properties have been proved. The quality of soybean proteins is comparable to that of animal protein sources.

The second oilseed protein in the oilseed meal market is canola/rapeseed where the application of protein isolates and/or concentrates in food products are still limited.

Cereal proteins

Cereal grains contain relatively little protein compared to legume seeds, with an average of about 10–12% dry wt. Nevertheless, they provide about three times the amount of proteins derived from the more protein-rich (20–40 wt%) legume seeds for the nutrition of humans and livestock (Shewry & Halford, 2002). As a plant source protein, wheat gluten ranks second to soy-based protein in terms of volume, and has grown steadily in production worldwide from 90,000 tonnes in 1980 to about 850,000 tonnes in 2008.

Legumes

Main sources include: beans, lupines, and peas. Peas are used most extensively as a source of commercial protein, fibre and starch. They are one of the more economically viable pulses to fractionate, they are grown extensively all over the world and the hull is easy to remove.

Leaf proteins

Examples are: alfalfa (Lucerne), grass, sugar cane, clovers, etc. Currently, there are no leaf protein raw materials in mass production commercially available, however, in Europe there are several initiatives and developments at the moment.

" New" sources

- Amaranth and quinoa produce significant amounts of edible grain, especially amaranth. The protein content of amaranth is about 16 wt% and its nutritional quality is also very high in comparison to cereals and some legumes. Amaranth proteins have high contents of lysine (twice that of wheat and three times that of maize), arginine, tryptophan and sulphur-containing amino acids (Shela Gorinstein, 2002).

- Roots and tubers: Proteins from roots and tubers have recently drawn increasing attention. One example of a protein source in this group is potato. On a dry weight basis, the protein content of potato is comparable to that of cereals.

- Marine and micro-organisms: Proteins can also be obtained from macro-algae, such as green and blue-green seaweed (e.g. *Spirulina* and *Chlorella*) and micro-organisms (e.g. mycoprotein, the key ingredient of the Quorn™ versatile meat-free product range).

- Single cell proteins: These are dried cells of microorganisms, like algae, fungi, yeast and bacteria, which are used as protein supplement in food or feed. In food they are used as aroma carriers, vitamin carriers, emulsifying agents, and to improve the nutritional value. Replacement of conventional protein sources is restricted because of their high nucleic acid content and slower digestibility.

PROTEIN REQUIREMENTS

Nutritional value and digestibility

One of the main reasons why plant proteins, as compared with animal proteins, are still limitedly used in food products is their lower nutritional value. Most animal-based proteins provide essential amino acids in balanced proportions; while many plant-based proteins provide them in less optimal proportions.

In most plant proteins, the concentration of lysine, one of the essential amino acids, is lower than that in animal proteins. Besides, the sulphur containing amino acids (methionine and cystine) are also relatively lower in legumes compared with amounts found in animal proteins, such as dairy, eggs and meat.

One of the factors determining the overall nutritional quality of dietary proteins is the amino acid content. The PDCAAS (Protein Digestibility Corrected Amino Acid Score) rates the nutritional quality of some plant proteins, e.g. those derived from soy and canola, to be equivalent to animal proteins (FAO/WHO, 1991). A PDCAAS of 1.0 indicates that soy proteins meet the protein requirements for human consumption when consumed as a sole source and the quality of soy proteins is comparable to that of animal protein sources such as milk (casein) and egg. Other plant proteins have comparatively low PDCAAS scores. (Day, 2013). Well-chosen mixtures of plant proteins can serve as complete and well-balanced sources of amino acids that meet the recommended human nutritional requirements (Table 4).

Pairing or combining various type of plant proteins is one way to compensate the unbalanced amino acid profile and low nutritional quality of individual plant proteins. Proteins from oilseeds or legumes that are low in sulphur-containing amino acids or proteins from nuts that are a source of lysine can be combined with most of the proteins from cereal grains which are deficient in lysine. For example, the PDCAAS of sorghum-based foods could be improved by supplementing with cowpea flour (Joseph O. Anyango, 2011).

Other factors determining the overall nutritional quality of dietary proteins are the digestibility and availability of the protein. The digestibility of plant proteins in their natural form is, in general, lower than the proteins from animal sources (WHO/FAO/UNU, 2007). During processing treatments, factors such as temperature, duration of heating and the amount of moisture may reduce the digestibility of proteins. For example, kafirin proteins in sorghum form extensive disulphide crosslinks and non-disulphide interactions when they are heated, resulting in poorer digestibility compared with the proteins of other similarly processed cereals like wheat and maize (KG Duodu, 2003).

If the dietary protein supply is equal to or above the recommended protein intakes, all of the essential amino acids from plant sources, including those sulphur-containing amino acids (in legumes) and lysine (in cereals) are considered as more than adequate or even exceed adult requirements, see Table 5 (WHO/FAO/UNU, 2007). However, for infants and growing children, who have relatively higher requirements of essential amino acids, compared with adults, this may not be the case. In the first year of our life and for growing children, a dietary protein supply containing balanced amino acids and easily digestible protein quality is essential.

Table 4. *Protein Digestibility Corrected Amino Acid Score (PDCAAS) values of individual plant proteins and examples when combined, compared with selected animal proteins (WHO/FAO, 1991) (Day, 2013)*

Protein	PDCAAS value
Casein	1
Egg white	1
Beef	0.92
Whole wheat	0.42
Wheat gluten	0.25
Rice	0.47
Maize	0.46
Sorghum	0.20–0.30
Soy protein concentrate	1
Pea protein concentrate	0.73
Chickpeas	0.71
Canola protein concentrate	0.93
Examples of combined plant proteins	
Wheat flour + canola meal (50:50 protein)	0.67
Wheat flour + pea flour	0.82
Wheat flour + soy protein	0.72
Rice + peas	1
Sorghum + cowpea	0.35–0.60

Amino acid compositions of some typical plant-based proteins

While soy and canola proteins have well-balanced amino acid compositions (see Table 5) that identify them as high quality proteins, flax and hemp proteins contain a high level of arginine, a precursor for nitric oxide production that may have an effect on immune response and muscle repair and growth (giving them unique characteristics) (Arntfield, 2011).

Table 5. *Amino acid composition (mg/g protein) of plant proteins from various cereal grains, legumes and oilseeds (Food and Agriculture Organisation, www.fao.org), (Day, 2013)*

Amino acid composition [mg/g protein] of plant proteins from various cereal grains, legumes and oilseeds in relation to the adult indispensable amino acid requirements (WHO/FAO 2007)

Essential amino acids	**Adult indispensable amino acid requirements**	Wheat (grain)	Barley (whole seed, de-hulled)	Maize (whole meal)	Rice (milled polished)	Sorghum	Soybean	Lupine	Pea	Chickpea	Canola
Arginine		48	50	43	79	34	73	98	102	98	58
Histidine	**15**	24	22	28	24	22	26	27	25	28	31
Isoleucine	**30**	34	38	38	44	41	46	45	46	46	23
Leucine	**59**	69	71	128	86	138	79	74	73	78	71
Lysine	**45**	30	37	27	38	21	65	55	81	71	56
Methionine	**16**	16	18	20	22	14	13	8	10	11	21
Cystine	**6**	26	24	16	16	16	13	14	12	12	24
Phenylalanine	**38**	47	54	50	50	51	50	38	49	60	38
Tyrosine		31	33	39	33	28	32	37	29	31	32
Threonine	**23**	30	35	37	34	31	39	38	44	39	44
Tryptophan	**6**	11	16	7	27	13	13	10	10	9	13
Valine	**39**	46	53	50	60	52	49	42	51	47	55

Gluten is one of the most important cereal proteins. It is the dough-forming protein of wheat flour – the key to the unique ability of wheat flour to form leavened products. Legume seeds are an abundant source of proteins, and lupine is one of the richest (about 35% of the dry weight).

Among the lupine seed species, blue and yellow lupine seeds are mostly used for feed, while the white lupines are primarily grown for food uses. White lupine seeds have a biological value of 91% of egg proteins (Egaña, Uauy, Cassorla, Barrera, & Yañez, 1992).

Potato is one of the most common staple crops in the world. Proteins from potato contain a higher proportion of lysine, which is often lacking in other plant protein sources. Potato proteins are, therefore, considered as high quality protein (A. Waglay, 2014).

Some algae contain very high level of proteins (up to 60% of total dry matter). Algae proteins have a high content of valine, leucine, lysine and phenylalanine, and a low content of sulphur-containing amino acids (cystine and methionine). In general, blue-green algae, including Spirulina, are highly digestible, and therefore do not require special processing. The nutritive value of algae proteins is comparable or even greater than that of most conventional protein feed supplements in term of protein content, amino acid quality and composition, biological value, nutritional acceptability, digestibility, and bioavailability of these nutrients

APPLICTIONS AND MARKETS OF ISOLATED " PURE" PROTEINS

Most protein containing products (e.g. those mentioned in Figure 1) are being consumed as such. A small group of proteins, however, is being extracted and used as protein concentrates and isolates. These protein products are mainly used in food products. The amount of proteins being used in the food industry is much lower in relation to the amount of protein containing biomass that is being used in the feed industry. Soy protein is dominating the food market, also in terms of prices. Pea protein is being produced as an alternative for soy protein. Further, wheat gluten and potato proteins are isolated as a side product of the starch industry. In addition to these plant proteins a number of proteins from animal origin is being produced, such as casein, gelatine and whey protein.

For food applications, the most important functional properties of a protein are its (water) solubility, water and/or fat binding/holding capacity, gel forming and rheological behaviours, emulsifying and foaming ability. Also the nutritional value is important. Many plant-based proteins are poorly soluble; consequently, their functional properties are still yet to be fully investigated.

Solubility

Solubility of a protein in an aqueous solution is often necessary for its functionality as an emulsifier and/or foaming agent. Its solubility is influenced by factors, such as: pH, ionic strength, solvent and temperatures. In general, protein isolates from soy, pea and lupine have good solubility at neutral pH. For proteins with high content of prolamins and glutelins (e.g. cereals), low solubility at neutral pH results in limited applications (except in dough-based products). One of the strategies to induce possible applications of plant proteins in food products is to investigate the possibilities to improve their solubility. Subsequently, there will be more possibilities for further investigations on their functionalities.

Emulsification

Research on the interfacial behaviours of (soluble) plant proteins is still limited. In general, plant proteins, with their larger molecules and structural constraint by disulphide crosslinks, form a relatively thicker layer at oil-water interfaces (Benjamin T. Wong, 2012). Their structure and the lack of structural unfolding upon adsorption may enable them to form protein particulates at the interface, which in turn, result in better emulsion stability compared to the emulsions stabilized by nano or micro-particles (Dickinson, 2012).

Foaming

While native plant proteins, with their compact structure, have limited foaming properties; albumin-rich plant protein fractions (e.g. from peas and lupines) have shown similar foaming properties to that of egg white proteins (S. Alamanou, 1997). The interfacial and rheological properties of proteins in general, and plant proteins in particular, can be affected by structural modification, either by changing pH or limited enzyme hydrolysis (Day, 2013).

Gel forming

Most proteins form heat-induced gels where the proteins, during heating, unfold, aggregate and rearrange further into a three-dimensional network that becomes stabilized upon cooling. The resistance of a protein against unfolding may be affected by the covalent interactions (e.g. disulphide bridges) stabilizing the gel network and environmental parameters, such as: pH, ionic strength and water availability. The gel formation mechanism of plant proteins containing globular fractions (soy, lupine, peas) is similar to that of common globular proteins where gel is formed as a result of protein denaturation (by heat) in aqueous solutions and the balanced interactions protein-protein and protein-solvent interactions (Day, 2013).

COMMERCIALLY AVAILABLE "PURE" PROTEINS

In Table 6 an overview is given of the prices of proteins that are commercially available. Formulated protein products, in which mainly hydrolysed proteins are being used, are not shown. The diversity of these formulated hydrolysates is large and no indicative prices are available. Small amounts of specific proteins are being used in functional foods, but no information is available on volumes and prices.

Because of the dominancy of soy proteins in the market, it can be stated that the prices of most vegetable proteins (except those derived from grains) are coupled to the soy prices. Exceptions are potato protein with food quality and zein. The traditionally produced potato protein is, by the lack of functionality, only being used in feed. Adaptations to this process generates potato protein with very high food related functionality. Zein is the alcohol soluble part of maize gluten. Also in this case, the bulk, corn gluten meal, is only being used in feed. A novel, mild process results in zein that is being used in niche markets, such as coating material for medicine tablets.

In general, the prices of animal proteins are higher than the prices of plant proteins. For example, the prices of whey protein (80 wt% protein) and casein (90 wt% protein) are twice as much 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 their traditional use in food products.

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.

Table 6. *Prices [€/kg] of isolated protein*

Vegetable proteins		**Animal derived proteins**	
Soy meal (40 wt% protein)	0.8	Gelatine (low quality)	2
Soy concentrate	2	Gelatine (high quality)	4-6
Soy isolate	3	Collagen	4-6
Pea concentrate	2	Egg white powder	6-8
Pea isolate	3	Egg yolk powder	4-6
Lupine concentrate (55 wt% protein	2-4	Plasma powder	3.5-4.5
Potato protein (feed)	0.8	Haemoglobin powder	0.7-1
Potato protein (food)	>>3	Milk powder	2.2
Wheat gluten	1.2	Whey concentrate (30 wt% protein)	1.6
Maize gluten feed	0.12	Whey concentrate (80 wt% protein)	5.5
Zein	25	Casein/caseinate	6.5

Table 7. *Worldwide use of isolated proteins in human nutrition*

Type of protein	**Amount [ktonne]**
Egg albumines	50
Whey proteins (WPI/WPC)[1]	80
Soy protein	210
Soy concentrate	360
Casein (caseïnate)	250
Gelatine	110
Wheat gluten	430

[1] Whey protein isolate (WPI) en whey protein concentrate (WPC)

PRODUCTION PROCESSES

In Table 6, the vegetable and animal derived protein products that are commercially on the market are shown. Vegetable proteins on the market are soy proteins, pea proteins, lupine protein, potato protein, wheat gluten and corn proteins.

In Figure 3, 4 and 5 respectively, the isolation processes of soy protein, wheat gluten and pea protein are depicted.

Soy protein

Soy beans have a high protein content and are converted into different soy protein products for use mainly in the food industry. Soy proteins have been produced for many years because of their nutritive value and well-balanced amino acid composition. In Figure 3, the production of the different soy protein products is being depicted starting from soy beans. The beans are dried, cracked, de-hulled and rolled into flakes. These flakes are either milled to obtain full fat soy flour or extracted with hexane to remove the oil. From the defatted meal the soy concentrates and isolates are being produced.

To increase the protein levels in soy protein products it is necessary to remove some other soy components than oil, resulting in soy concentrates with protein content of about 70 wt% and isolates with a protein content of about 90 wt%. Soy concentrates are prepared from defatted flakes by removing a major part of the sugars and the minerals. Soy isolates are produced by extraction of flakes at high pH followed by isoelectric precipitation.

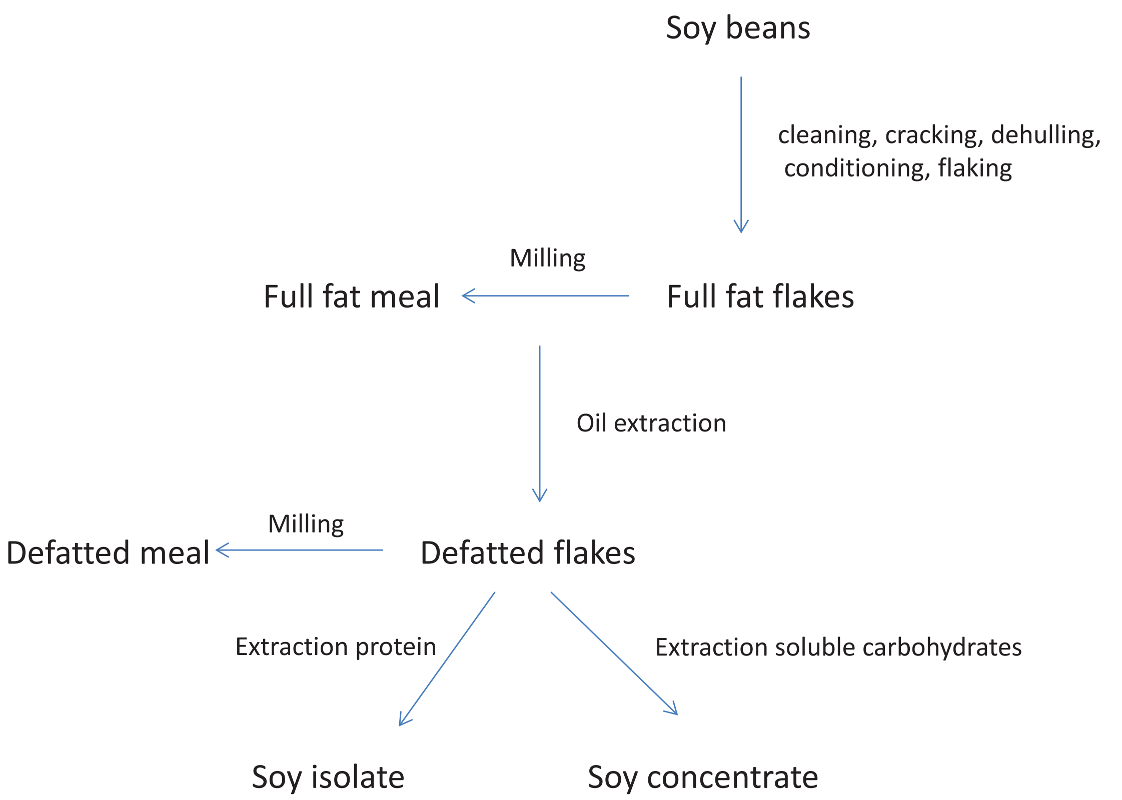


Figure 3. *Process scheme production soy beans*

Wheat gluten

Wheat flour is used in a wide range of bakery products, such as: bread, pastries, biscuits etc. Wheat gluten has been produced since decades as a by-product of the wheat starch manufacturing. Wheat gluten is a rather cheap protein product, and is being used in several food related formulations.

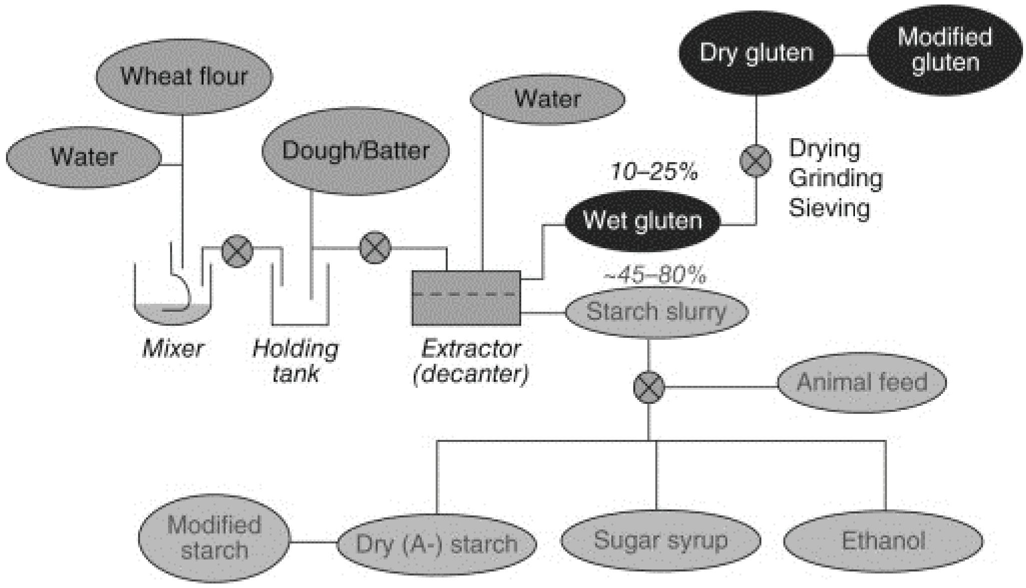


Figure 4. *Flow chart of a type of gluten/starch separation and production process*

The poor solubility of wheat gluten in water, and its cohesive properties are used to separate it from wheat starch (Figure 4). First a dough is produced that is diluted to a suspension. In this suspension gluten aggregates start to form larger particles and separate physically from the starch. Next, the starch slurry is separated by screens, hydro-cyclones or decanters from the gluten particles that form in this step a large cohesive mass. The wet gluten is washed and dried in a ring dryer developed specially for this use. Here, gluten is chopped and fed as small wet particles into a stream of dry gluten particles circulated by hot air. The drying step is the most critical step for the gluten quality or its "vitality". Wheat starch is washed and purified in hydro-cyclones, concentrated in decanters, and subsequently dried or modified. Most of the process water is recycled.

Pea proteins

The increasing need for protein-rich raw materials for both animal feed and new products for human nutrition have led to interest in pea as an interesting crop as being favoured by the EU. Like soy protein, pea protein shows the advantage of a well-balanced profile of amino acids.

Pea protein enriched products are prepared for food uses either by a dry or a wet process. Using the dry process, the pea seeds are being milled and the starch and proteins are being separated by air classification. The products have a protein content between 50 and 60 wt%.

The wet process is based on the solubility properties of the pea proteins (Figure 5). The proteins are solubilised by dispersion the flour in water and pH adjustment. The protein extract is clarified by use of cyclones or centrifugation and the proteins are recovered by ultra-filtration of iso-electric precipitation. These isolates have a protein content of about 85-90 wt%.

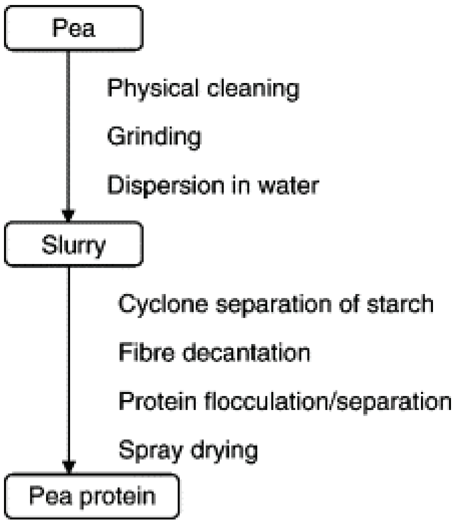


Figure 5. *Preparation of a pea protein isolation, commercial operation, adapted from data provided for Nutralys® pea protein (Roquette, 2010)*

Potato and corn proteins

In the potato industry the potatoes are ground and the insoluble material, mainly starch and cell wall material, is removed by hydro-cyclones. The remaining fluid is the fruit juice. The proteins can be recovered by a combined acidic heat treatment of the potato fruit juice. This treatment results in irreversible precipitated proteins, which have lost important functionalities, such as water solubility. Therefore, potato protein can only be used as animal feed. Solanic, however, has developed a process by which potato protein is being obtained with high functionality (see chapter 4).

The basic processing of corn is being carried out by the wet milling technology yielding corn oil, corn fibres and corn starch. In the U.S. the corn starch fraction is being used as such or transferred into corn syrup, dextrose or bioethanol. Wet milling of corn also generates corn gluten meal. An important step in the wet milling is the so called the steeping process, by which a sulphite solution is being used to soften the maize kernel. This process step has the drawback that the proteins present in the cakes have lost the majority of their functional properties and, therefore, can only be used as feed.

MARKET TRENDS OF APPLICATIONS IN FOOD PRODUCTS

For the consumers, protein is not only part of a healthy diet and a source of energy but also has benefits on muscle and weight maintenance. About one-fourth of the U.S. consumers have increased their protein intake over the past two years. Besides, the growing world's population leads to increased demand for proteins.

Being the most established and researched protein group, dairy proteins have extended their application in new product categories, such as: spreads, ready meals, and purees. 5.2% of global launches in 2014 contained a dairy protein (4.8 % in 2013). The rise of alternative proteins, in particularly sustainable plant-based sources is showing no signs of slowing down.

Innova Market Insights revealed a 24% growth (2014 vs. 2013) in global high-protein launches with plant-based proteins. While soy protein concentrates and isolates are still the leading proteins used, the number of newly launched food products containing plant protein from other sources is increasing (see Figure 6). Application of pea protein in food products has grown. In 2014, almost 12,000 food products with plant-based proteins in the ingredient list were launched worldwide (Innova Database, 2015). The use of plant-based proteins as animal protein substitutes has increased the number of product launches with a vegetarian positioning, and product claims such as "vegan friendly", "suitable for vegans" or "free from..." (e.g. gelatine free, dairy free and egg free).

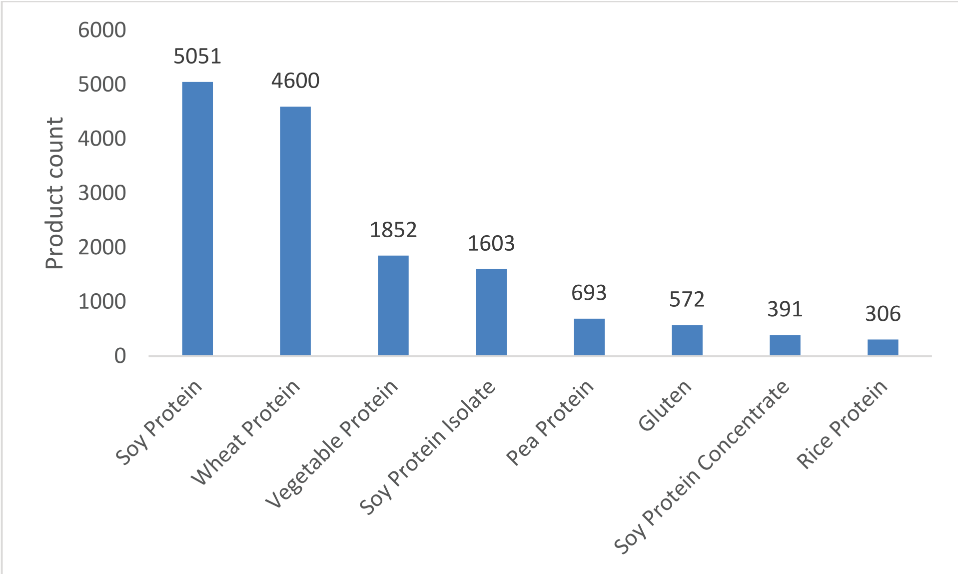


Figure 6. *Number of newly launched products (worldwide) containing plant-based proteins in 2014 (Innova Database, 2015)*

The first commercial products made with proteins from insects have been introduced. Even though insects are sustainable (require less water, land and feed than other animals) and high in valuable nutrients (rich in amino acids, calcium and vitamin B), consumer's acceptance, cost, texture and appearance is still a challenge for mass production.

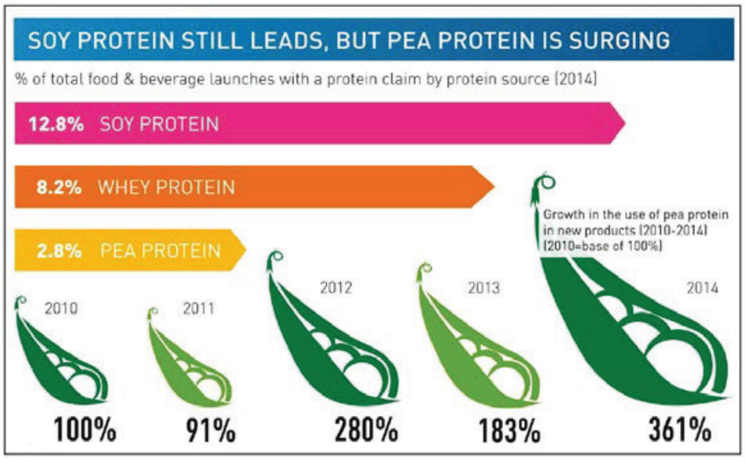


Figure 7. *Growth in the use of pea proteins in newly launched food products (2010 - 2014) (Innova - Food and Beverage Innovation, 2015)*

Plant-based proteins have also gained a lot of attention in scientific research and patents. The number of patents on nuts and seeds has grown from 8 in 2010 to 204 in 2013 (see Figure 8).

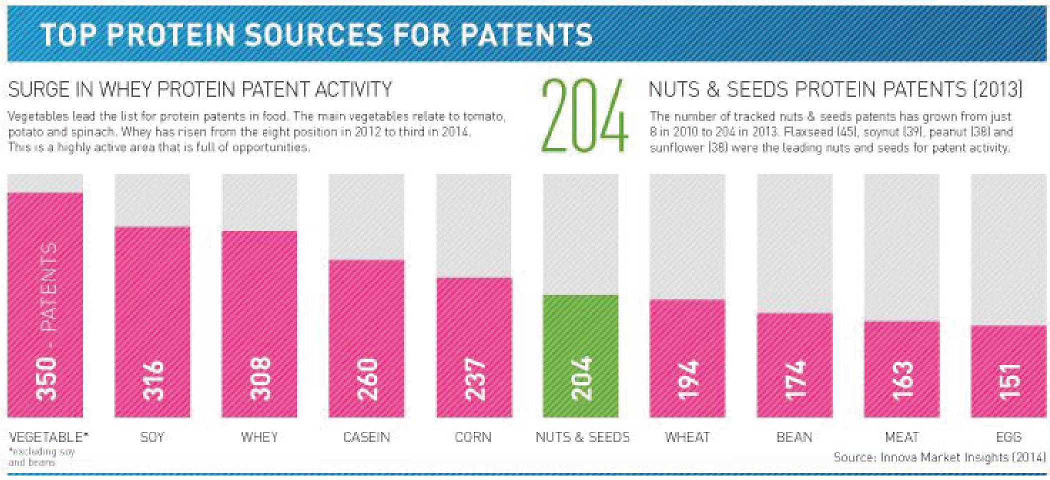


Figure 8. *Top protein sources for patents (2014) (Innova Database, 2015)*

CHALLENGES

Solubility

For applications in food products, solubility of a protein in water is one of the most important characteristics determining its success. Water solubility is a key parameter determining some of the important functionalities of a protein, such as: foaming, emulsifying, gelling and water holding capacity (A. C. Alting, 2011). <u>The poor solubility of plant proteins in water, consequently limited functionality, is therefore a limiting factor for their applications.</u>

In 1924, Osborne has been the first to classify plant storage proteins based on their solubility and extractability in various solvents. The "Osborne fractions" include four major classes of proteins: albumin, globulin, prolamin, and glutelin (Table 8). Based on the Osborne fractionation, for example, the maximum amount of water-soluble (functional) proteins that can be extracted can be addressed. Nowadays, it has been recognized that each of these solubility classes contains a complex mixture of proteins and overlaps between the classes exist (Day, 2013).

Albumins are compact globular proteins consisting of two polypeptide chains linked by a disulphide bridge. They contribute more than 50 wt% of the total sulphur in the seeds of legume, such as peas and lupines. They are present in small quantity in cereal seeds but more in oilseeds and legume.

Globulins contain low levels of sulphur containing amino acids (cysteine and methionine). They are the major protein fraction in legume, such as: soybeans, lupines, and peas but low in cereals.

Prolamins are high in content of proline and glutamine. They are the major storage proteins in cereals (about 50 wt% of the total grain proteins, except for rice: approx. 4 wt%). Common prolamin proteins are gluten in wheat, hordeins in barley, and zeins in maize.

Glutelins have high molecular weights (45 to 150 kDa). They contribute up to 80 wt% of the total rice protein. Their extensive aggregation, disulphide bond crosslinking and glycosylation cause difficulties in extraction and consequently, limited investigation.

Table 8. *Osborne fractions of plant storage proteins*

Albumin	Water-soluble, heat-coagulatable
Globulin	Water-insoluble, saline-soluble
Prolamin	Water/saline-insoluble, extractable in concentrated aqueous alcohol solutions (60-70 % v/v)
Glutelin	Water/saline/alcohol-insoluble, extractable in dilute aqueous acid or alkali solutions

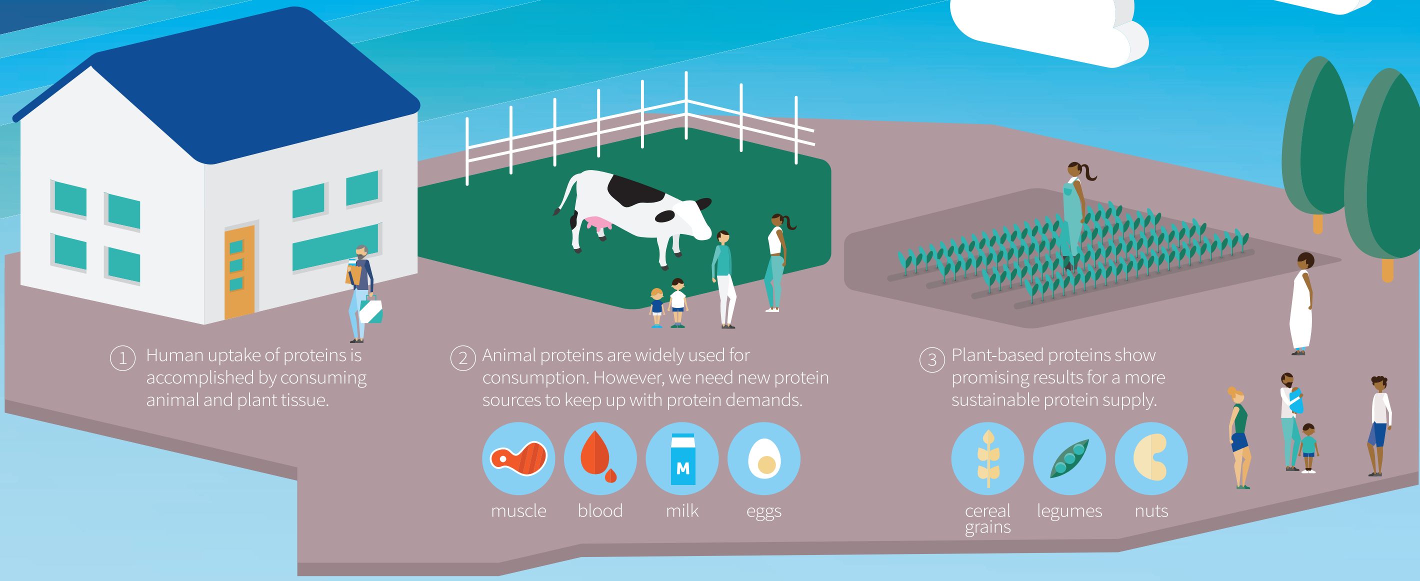
Table 9. *Approximate distribution of the different classes of proteins from different plant sources, according to the Osborne classification (Osborne, 1924; Shewry & Casey, 1999)*

Plant source	Albumins [wt%]	Globulins [wt%]	Prolamins [wt%]	Glutelins [wt%]
Wheat	6–10	5–8	35–40	40
Rice	2–6	12	4	80
Barley	3–5	10–20	35–45	35–45
Maize	4	4	60	26
Sorghum	2–7	2–10	35–60	20–35
Soybean		90		
Pea	15–25	50–60		
Chickpea	8–12	53–60	3–7	19–25
Lupine	25	75		
Canola	20	60	2–5	15–20

Allergenicity of proteins

Many plant-based proteins do not only contain various anti-nutrients but are also identified as allergens causing allergic reactions in 1-2% of the population. Allergenic proteins from soybeans are identified as major allergens. Allergenic responses to some proteins from legumes have been reported. Wheat gluten and similar proteins from barley and rye do not only have allergenicity but also is linked to Coeliac disease of which the prevalence in adult populations fluctuates between 1 in 100 and 1 in 300 people in most of the world (Dana Flodrová, 2015). Proteins from rice, maize and barley may give rise to allergic reactions, such as atopic dermatitis and asthma (Shela Gorinstein, 2002). Pea proteins have low allergenicity and the consumption of potato has showed the lowest incidence of allergenicity for both vegetable and animal protein sources. (Handbook of food proteins, 2011; Day, 2013).

Plant-based proteins could meet humans' growing demand for protein



Plant vs. animal

Plant proteins are still limitedly used in food products, compared to animal proteins.

This is due to their lower nutritional value.

Market prices

Market prices of animal proteins are twice as much than those of plant proteins.

animal protein

plant protein

Pairing plant proteins

Pairing or combining various type of plant proteins is one way to compensate the unbalanced amino acid profile and low nutritional quality of individual plant proteins .

plant proteins with low nutritional quality + plant proteins with low nutritional quality → combined plant protein with high nutritional quality

PDCAAS is a method of evaluating the protein quality based on the amino acid requirements of humans and their ability to digest it. 1 = high 0 = low.

animal protein		plant protein		combined plant protein	
1	Casein, Egg white	1	Soy protein concentrate	1	Rice + peas
0,92	Beef	0,93	Canola protein concentrate	0,82	Wheat flour + pea flour
		0,73	Pea protein concentrate	0,72	Wheat flour + soy protein
		0,71	Chickpeas	0,67	Wheat flour + canola meal
		0,47	Rice	0,35	Sorghum + cowpea
		0,46	Maize		
		0,42	Whole wheat		
		0,25	Wheat gluten		
		0,2	Sorghum		

PDCAAS (scale 1 – 0)

Challenges plant-based proteins

Solubility of a protein in water is one of the most important characteristics determining its success.

Many plant-based proteins contain anti-nutrients and allergens causing allergic reactions in 1-2% of the population.

Trends

Market trends of application of plant protein in food products.

1. More focus on a heatlty diet and weight maintenance.
2. Dairy products are the most established and researched protein group and continue to expand their application in new product categories.
3. The world population is growing, so demand for proteins increases.
4. Alternative proteins, particularly plant-based sources, are on the rise.
5. Commercial products made with insect protein have been introduced.

Plant-based proteins could provide new and sustainable food ingredients for humans to meet the growing demand for protein

4. Protein products for compound feed

PROTEIN CONTAINING CROPS USED IN FEED

The European and global feed production from 2005 till 2012 is presented in Figure 9. (Fefac, 2013). The European feed production increased by 23% from 2005 till 2012, the global feed production by 47%. The prospect is that 1500 million tonnes of feed will be produced in 2050 (Fefac, 2013).

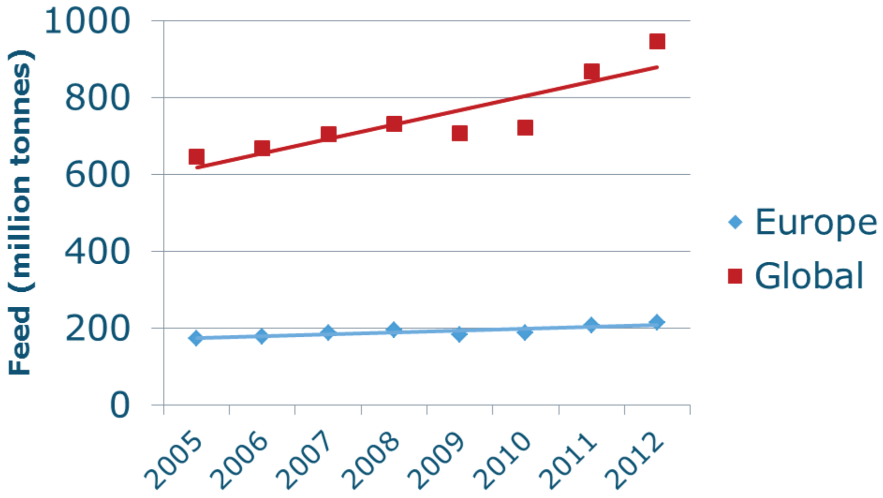


Figure 9. *The European and global feed production from 2005 till 2012 (Fefac, 2013)*

This increase in feed production also means that the need for protein sources in feed is increasing. Protein containing crops are the main (traditional) protein sources in feed. Milk protein and fishmeal are only used in diets for young pigs. Potential new protein sources in feed for pigs and poultry are leaf proteins, aquatic proteins and insects. The main protein containing crops and potential new protein sources for pigs and poultry are presented in Table 10. (adapted from Van Krimpen et al., 2013).

Oil seeds

The oil seed crops (soybeans, rapeseed and sunflower seed) are grown for their oil content but the protein content of these seeds is also relatively high. The defatted fractions are generally used in feed.

Soybeans have a very high protein level of about 40 wt% and a relatively high fat level (20 wt% oil). They are processed for the production of soybean oil and soybean meal, both of which are used in feed. Soybeans are mainly produced in USA, Argentina, Brazil, China and India. Only a small amount is cultivated in Europe. For the production of Dutch feed 1.8 Mt of soybeans is used (Van Krimpen et al., 2013). Soybeans produce significantly more protein per hectare than other large oilseed crops: 940 kg/ha, versus 792 kg/ha for rapeseed and 280 kg/ha sunflower seed (Vahl, 2009).

Rapeseed has a relatively high protein level of about 23 wt% and a high fat level (40 wt% oil). The oil is used for human consumption and as biodiesel. Rapeseed meal contains about 35 wt% protein (CVB, 2012) and is used for animal feed. In Europe, rapeseed is mainly produced in France and Germany.

Sunflower seed has a relatively high protein level of about 23 wt% and a high fat level (40 wt% oil). The oil is used for human consumption. Sunflower seed meal contains about 35 wt% protein (CVB, 2012) and is used for animal feed. Sunflowers are not cultivated in the Netherlands.

Table 10. *Main protein containing crops used in feed and potentially new protein sources*

Category	Protein source
Traditional protein containing crops	
Oil seeds	Soybeans, rapeseed and sunflower seed
Grain legumes	Peas, Vicia faba, lupines
Cereals and pseudo cereals	Wheat, barley, oat, maize, quinoa
Leafs	Grass
Potential new protein sources for pigs and poultry	
Forage legumes	Lucerne (alfalfa)
Leafs	Grass, beet leafs, other herbaceous agro-residues
Aquatic biomass	Algae, both seaweed and microalgae, duckweed
Insects	Mealworm, housefly, house cricket
Single cell proteins	Algae, fungi, bacteria, yeast

Grain legumes

Grain legumes (peas, *Vicia faba* and lupines) are cultivated for their seeds. The seeds are used for human consumption, for animal feed, and for the production of oils for industrial use.

Pea is the most cultivated grain legume in Europe and has a protein content of about 20-25 wt% (CVB, 2012). The cultivated area of peas in the Netherlands is relatively small (Kamp et al., 2010). The yield in the Netherlands is 5-6 ton/ha (Van Krimpen et al., 2013).

Vicia faba is the second most cultivated grain legume in Europe and has a protein content of about 25 wt% (CVB, 2012). The cultivated area of *Vicia faba* in the Netherlands is relatively small (Kamp et al., 2010). The yield is 5-6.5 ton/ha (Van Krimpen et al, 2013).

Lupines have a protein content of about 35 wt% (CVB, 2012). The cultivated area in the Netherlands is small (Kamp et al., 2008). The yield is 1-5 ton/ha (Van Krimpen et al., 2013).

Cereals and pseudo cereals

Cereals are used for human consumption and for animal feed. Cereal grains like wheat, barley and oat contain about half of the protein content of Legume grains (12-15 wt%).

In feed they are mainly used as an energy source but they also deliver part of the protein. The yield per ha of wheat, barley and oat is 7-10, 6-8 and 3-5 ton/ha, respectively.

Pseudo cereal quinoa has a protein content of about 15-18 wt%. It is mainly used for human consumption. It is not common practice to use it in feed. The current knowledge of the nutritive value of Quinoa is not sufficient for accurate inclusion of this ingredient in feed. Quinoa is not cultivated in the Netherlands.

Leafs

Grass is mainly used for ruminants but it is also used for pigs and poultry in organic pig and poultry husbandry. The yield is expected to be 10-15 tons dry matter per ha (Van Krimpen et al., 2013). Van Krimpen et al. (2013) concluded that grass can to some extend contribute to the protein supply of pigs. The fibre content of these ingredients, however, will limit their use in diets for pigs. Biorefineries might increase the possibilities to use grass by separating the protein and fibre fractions, but techniques should be further developed for application in practice. Van den Pol-Dasselaar et al. (2012) concluded that grass refining has potential but still a large number of questions, including ecological and societal ones, have to be addressed. Moreover, further development of protein extraction techniques is necessary to increase protein yield and to make these techniques economically feasible.

Forage legumes

Lucerne (alfalfa) is the most cultivated forage legume in the world and is mainly used for animal feed. Lucerne is mainly produced in the USA, Canada and Argentina. In Europe it is mainly produced in France and Italy. The protein content of Lucerne is about 18 % per kg dry matter (CVB, 2012). The yield is about 8 ton/ha (Van Krimpen et la., 2013).

Aquatic biomass

Aquatic protein sources are algae, seaweed and duckweed. There is no commercial algae, seaweed or duckweed cultivation on a large-scale available yet in Europe.

Algae can contain large amounts of proteins (25-50 wt%) depending on the strain used (Becker, 2007; Mulder, 2010). In growth experiments and pilot plants, yields could be reached of 15-30 tons dry matter per ha per year (Van Krimpen et al., 2013). Results from literature suggest that algae can be a useful protein source in feed. Further investigation, however, is needed regarding the composition, nutritive value and use of algae in animal nutrition (Christake et al., 2010). For the production of algal protein less land is necessary than for the production of traditional protein crops (Spruijt et al., 2014). High production costs might be a bottleneck for cultivation of micro-algae solely for protein production (Van Krimpen et al., 2013).

Seaweed is mainly used for human consumption and is produced in China and Japan. Depending on the species, seaweeds contain between 10-30 wt% protein (Mulder, 2010; Bikker et al., 2014). Bikker et al. (2014) studied the nutritive value and relevant characteristics of seaweed for use in animal diets. They concluded that there is a large variation in nutritive value between different species of seaweed. The in vitro ileal digestibility of seaweed was moderate (20% lower than soybean meal).

Duckweed has a protein content of about 35 wt%. Duckweed is a putative new protein crop with very high potential (high protein production per ha and it does not compete for arable land use), but it needs more (scientific) input on the level of cultivation, processing and the application in feed (Van Krimpen et al., 2013).

Insects

Insects contain between 30 and 70 wt% protein on a dry matter basis. Veldkamp et al. (2012) concluded that the use of insects as a sustainable protein rich feed ingredient in pig and poultry diets is technically feasible. Insects can be reared on low-grade bio-waste and can turn this bio-waste into high quality proteins. Main bottlenecks for use in the near future are in the area of legislation and the achievement of a low cost price by automation of the production process (Veldkamp et al., 2012). To introduce insects as a feed ingredient in the pig and poultry feed chain, additional research is recommended on its feeding value, inclusion levels in poultry and pig diets, functional properties of the feed ingredient, safety when using bio-waste as a rearing substrate, extraction of nutrients, shelf-life, and use of left-over substrates and residue products of insects (Veldkamp et al., 2012).

Single cell proteins

Single cell proteins have potential in feed as fattening agents for calves, poultry, pigs and fish.

PRODUCTION AREA AND YIELD OF PROTEIN CONTAINING CROPS

In Figure 2. (Chapter 2) the production of protein containing crops in the world is presented. In Table 11. the production area and the yield of the protein containing crops in Europe and in the Netherlands is presented.

The production area of grass is both in Europe and in the Netherlands much higher than the production area of other protein containing crops. Soybean is hardly cultivated in the EU and not in the Netherlands. The production yield in the different European countries in presented in Figure 10. In Europe soybeans are mainly produced in Ukraine and in Russia.

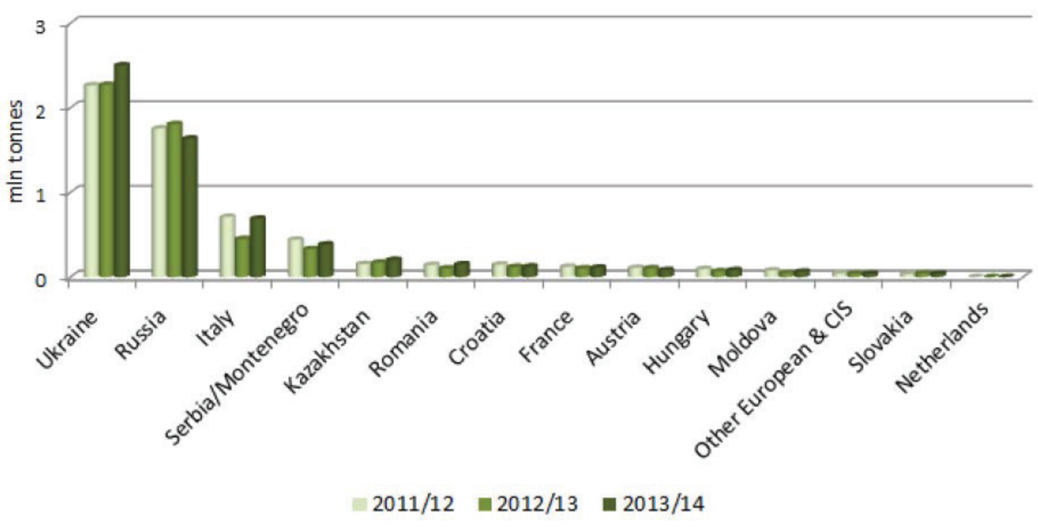


Figure 10. *Production yield of soybeans in Europa (source: Soy barometer 2014)*

Table 11. *Production areas and production yield in the Netherlands and in Europe for the different protein containing crops (Van Krimpen et al., 2013)*

	Area production in NL (ha x1000)	Area production in EU (ha x1000)	Yield in NL (tx1000)	Yield in EU (tx1000)
Oil seeds – soybean	-	2,740	-	4,790
Oil seeds – rapeseed	2.6	8,770	11.5	23,080
Oil seeds – sunflower	-	3,700	-	12,002
Legumes (pulses) – peas/beans/lupine	9.3	2,480	67.9	6,530
Legumes (pulses) - chickpea	-	60	-	80
Legumes (forage) – Lucerne	6.4	7,120	-	78,320
Cereals – oat	1.7	2,700	8	7,400
Pseudo cereals – quinoa	-	0.25	-	0.27
Leaves – grass	941	182,344	9,410*	1,823,440*
Leaves – (e.g. sugar beet leaves)	71	3,229	3,500*	149,800*
Macroalgae – seaweed	-	-	-	-
Microalgae	-	-	-	-
Duckweed	-	-	-	-

Sources: Eurostat (http://epp.eurostat.ec.europa.eu/portal/page/portal/statistics/search_database); FAOSTAT (http://faostat.fao.org); CBS StatLine (http://www.cbs.nl/en-GB/menu/themas/landbouw);
* estimation, because no data available

In Table 12. the protein content of the different protein sources, the yield per hectare in Europe, and the protein yield per hectare are presented. For the algae (micro and macro) and duckweed, the numbers are based on pilot cultivation trials.

Soybeans have a high protein content (40 wt%) but the yield per hectare in Europe is much lower than the yield per hectare of wheat, making soybean cultivation less attractive for farmers in Europe. Duckweed also has a high protein content. Peas, beans, rapeseed an sunflower seed have a relatively high protein content. The protein content of Lucerne and grass is not so high but because of the high yield per ha, the protein yield per ha is relatively high. The protein content of oat is comparable with that of wheat. The yield per hectare of oat, however, is much lower than that of wheat.

Table 12. *Protein content, yield and protein yield of various protein sources (Van Krimpen et al., 2013)*

	Protein content **[wt%]**	**Possible yield EU** **[tons ds/ha/y]**	**Possible protein yield** **[tons/ha/y]**
Oil seeds – soybean	40	1.5-3	0.6-1.2
Oil seeds – rapeseed	25	3	0.75
Oil seeds – sunflower	23	3	0.7
Legumes (pulses) – peas/beans/ lupine	17-35	4-6	1-2
Legumes (forage) – Lucerne	19	13	2.5
Cereals – oat	12-15	3-5	0.4-0.75
Pseudo cereals – quinoa	12-18	3	0.4-0.5
Leaves – grass	12	10-15	1.2-2
Leaves – (e.g. sugar beet leaves)	12	4.5	0.5
Macroalgae – seaweed	10-30	25	2.5-7.5
Microalgae	25-50	15-30	4-15
Duckweed	35-45	30-40	10-18
Wheat (as reference)	11	10	1.1

Cormont and Van Krimpen (2014) investigated the share of protein of regional sources in the total Dutch feed use for the years 2011 and 2013. They defined 'regionally sourced protein' as those ingredients that supply the animal feed with proteins, and that originate from crops grown in Europe. In 2013, the production of regionally and not regionally sourced protein was 3,314 and 1,042 ktonne, respectively. This means a share of protein of regional source in the total Dutch feed use of 76% in 2013. Not regional protein sources are mainly coming from soybeans (69%), sunflower seed meal (17%) and palm kernel (11%).

PRICES OF PROTEIN CONTAINING CROPS

The price development of soybeans from 2010 till 2015 is presented in Figure 11., the price development from soybeans compared to wheat in Figure 12.

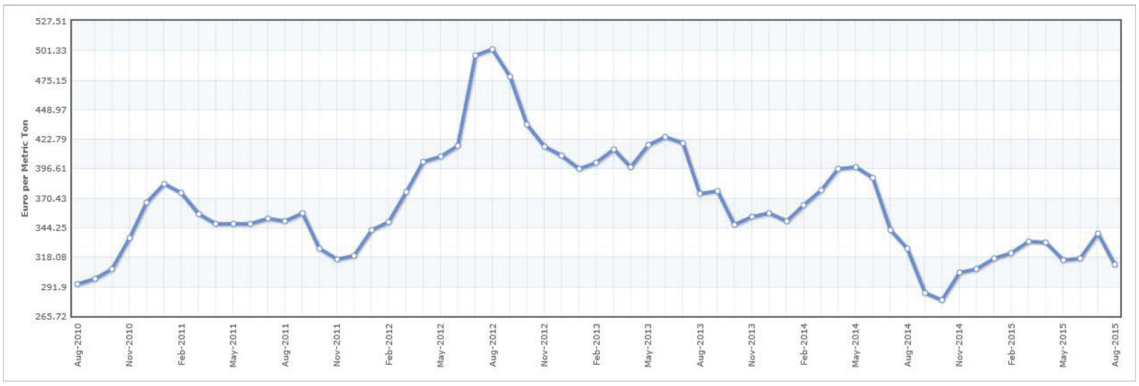


Figure11. *Price development of soybeans from 2010 till 2015 (www.indexmundi.com/commodities)*

The price of soybeans increased from 2010 till 2012, and then decreased till 2014. From 2014 to 2015 the price increased gradually. In 2013 and 2014 the harvest of soybean was high. The price development of soybeans is quite comparable with the price development of wheat from 2010 till 2015 (Figure 12.).

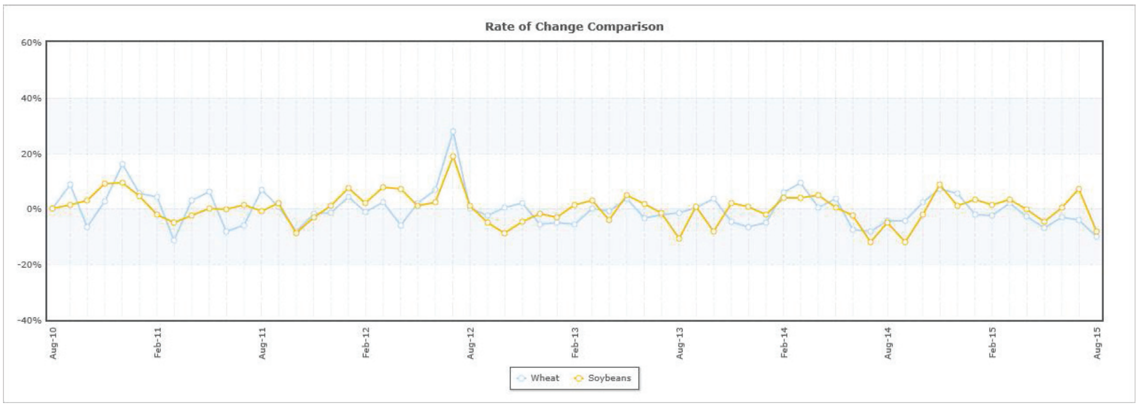


Figure 12. *Price development of soybeans compared to wheat from 2010 till 2015 (www.indexmundi.com/commodities)*

The delivered price of protein sources (delivered to the feed company) from July 2014 till June 2015 in the Netherlands is presented in Table 13.

Table 13. *Delivered price [€/kg] of various protein sources from July 2014 till June 2015 (www.voederwaarderprijzen.nl)*

Protein source	Price [€/kg]
Soybeans (toasted)	0.46
Soybean meal (CF[1]<45, CP[2]<480)	0.40
Rapeseed meal (CP<380)	0.23
Sunflower seed meal (CF<160, CP 380)	0.25
Palm pit kernel (CF<180)	0.14
Peas	0.25
Lucerne (CP 160-180)	0.19
Maize	0.17
Maize gluten meal	0.77
Wheat	0.17
Barley	0.17

[1] CF = crude fibre (in g/kg); [2] CP = crude protein (in g/kg)

The price of soybean meal is higher than the price of the other protein sources. The price of rapeseed meal is somewhat lower than the price of sunflower seed meal and of peas. Palm pit kernel has the lowest price. The differences in prices for protein containing biomass has several reasons, among others the protein content and the protein quality (digestibility, amino acid profile), yield per hectare, market volumes and transport costs. For example, palm pit kernel meal has a lower protein content and is therefore cheaper than rapeseed meal.

USE OF AMINO ACIDS

Apart from proteins, also amino acids are used as supplements in feed. The reason is that typical animal feed, like soybean meal, do not possess all the essential amino acids in sufficient amounts for pigs. The most limiting amino acid is usually methione, followed by lysine, threonine and tryptophan, and is therefore separately added to feed without having too high total protein content. Methionine is chemically produced but the other amino acids are produced via fermentation processes.

BOTTLENECKS IN USING PROTEIN CONTAINING CROPS IN FEED

Bottlenecks in using protein containing crops in feed have concerns in particular anti-nutritional factors (ANFs), cultivation aspects, legislation and costs.

Anti-nutritional factors

ANFs are compounds in protein containing crops which can negatively affect the nutritive value. The most important ANFs are: tannins, protease inhibitors, phytate, alkaloids, lectins, pyrimidin glucosides and saponins (Helsper et al., 2006).

Soybeans contain protease inhibitors and phytate (Stegeman, 2010). Protease inhibitors reduce the activity of proteases which are essential for digestion and absorption of protein. Protease inhibitors (e.g. trypsin inhibitor) can be inactivated by heat treatment or by fermentation (Helsper et al., 2006). Phytate can bind metals ions as zinc and iron and thus reduce the absorption of these trace elements. In addition, phytate can inhibit protein availability (Frederikson et al., 2001). Phytate-bound phosporus cannot be digested by pigs and poultry and will therefore be excreted with the faeces.

Rapeseed meal contains the ANFs erucic acid and glucosinolates. Glucosinolates can decrease the iodine uptake resulting in symptoms of iodine deficiency (Schöne et al., 1990), thyroid dysfunction, lowered fertility (Mawson et al., 1994), and growth depression. As a result of European breeding programs, the levels of erucic acid and of glucosinolates have decreased (Lamont and Lambrechts, 2005). Rapeseed extract with a level of erucic acid lower than 2 wt% and a level of glucosinolates lower than 25 μmol/g is called Canola (Canadian Oilseed, Low-Acid) or "rapeseed meal double-zero". The high levels of ANFs and of fibre in rapeseed meal makes it less valuable than soybean meal as a feed ingredient. Over the last few years canola protein concentrates with about 55 to 60 wt% crude protein have been available. Canola protein is extracted and fully denatured to render the protein insoluble. Soluble ANFs (like glucosinolates) are washed from the protein (www.canproingredients.ca).

Sunflower seed has a high content of phenolic compounds, mainly consisting of chlorogenic acid (0.5 - 2.4 wt% of dry matter) (Gonzalez-Perez et al., 2002). These compounds have the capability to interact with proteins, thereby reducing protein digestibility and decreasing the nutritional value for animal feed. It is technically possible to produce sunflower protein concentrates with a crude protein content of at least 75 wt% (Salgado et al., 2011). Sunflower protein concentrates could be of interest in pig and poultry diets. Until now, however, no information is available regarding nutritional values of these concentrates for monogastrics (Van Krimpen et al., 2013).

Anti-nutritional factors in pea are protease inhibitors (trypsin inhibitor) and phytate (Stegeman et al., 2010). White-flowering, round peas are used in a large scale for feeds. These pea types contain a little amount of tannins and are often low in trypsin inhibitor activity (TIA) (Helsper et al., 2006). Other pea types such as grey peas are less appropriate due to their high TIA. Cultivar choice is therefore very important (Helsper et al., 2006). The concentration of phytate is highly variable with the cultivar, differs between locations and depends on the maturity stage of the seed (Helsper et al., 2006). Soaking of pea meal at 45 °C is very effective to decrease phytate levels (Fredrikson et al., 2001). Over the last few years, pea protein concentrates have been available for use in pig and poultry diets. The concentrate contains about 80% protein and a low level of TIA (www.roquette.com).

Anti-nutritional factors in field beans are protease inhibitors (trypsin inhibitor), phytate and convicine/vicine. White-flowering Vicia faba has a low content of tannins and no condensed tannins and this allows a larger inclusion rate in animal diets for white-flowering cultivars than for coloured-flowered cultivars (Helsper et al., 2006). Convicine/vicine are specific for Vicia faba and they can disturb fat metabolism and fertility in laying hens (Helsper et al., 2006). However, there are Vicia faba genotypes available that are almost free of vicine/convicine.

The most important ANFs in lupine species are toxic alkaloids from which quinolizidin alkaloids are the most relevant (Liener, 1989). Alkaloids are bitter tasting and may reduce the feed intake. The blue and white lupines have much higher levels of alkaloids than the yellow species. Low-alkaloid varieties, also known as sweet lupines, are generally available (Van Krimpen et al., 2013).

In contrast to Vicia faba and peas, lupines contain hardly any trypsin inhibitor activity and only low levels of saponins (Helsper et al., 2006). Moreover, they contain very low protease inhibitor activity.

The main ANF in quinoa is saponin, but it also contains phytate, tannins and trypsin inhibitor activity (Ahamed et al., 1998). The bitter tasting saponins accumulate mainly in the seed coat and may be removed by soaking (Helsper et al., 2006). Breeding activities at Plant Research International (Wageningen, The Netherlands) have made saponin-free cultivars available. Although quinoa might have some promising nutritional properties, current knowledge of the nutritive value is not sufficient for accurate inclusion of this ingredient in diets of monogastrics.

The presence of ANFs in field beans, lupines, peas en quinoa are summarized in Table 14.

Table 14. *Anti-nutritional factors in field bean, lupine, pea and quinoa (Helsper et al., 2006)*

Crop species	Field bean	Lupine	Pea	Quinoa
Alkaloids	no	no, in alkaloid-free cultivars	no	no
Tannins	no, in tannin-free cultivars	no	no	yes
Convicine/vicine (only relevant for laying hens)	yes in most cultivars; no, in few cultivars	no		no
Phytate	yes	yes	yes	yes
Protease-inhibitors	yes	no	yes in most cultivars; no, in few cultivars	yes
Lectins	yes	yes	yes	yes
Saponins	yes	no	no	no, in saponin-free cultivars

Cultivation

Soybeans can fixate nitrogen due to a symbiotic relationship with Rhizobium bacterium strains. Cultivation of soybean is successful in regions with hot summers, with optimum growing conditions at a mean temperature of 20 to 30°C. The climate in North-West-Europe is less optimal for cultivation of soybean. The crop needs to be sawn before half of April in order to ripen in time, but night frost in April might harm the crop. Cultivation in Europe will need breeding for cultivars with a short growing season (Vahl, 2009). The relative low yield in Europe, in combination with a long growing season, enhances the water content of the beans, which is unfavourable for long storage of the beans (Van Krimpen et al., 2013). This makes soybean cultivation in the Netherlands less attractive than cultivation of peas and beans.

Peas are very sensitive to pathogens and pests, and the plants lodge (fall down) easily. Different fungal diseases, insects, birds and weeds can attack the crop. Once in the soil, fungal species may initiate disease development for many years thereafter. The damage may be considerable and the entire crop may be lost for that season. Therefore, crop rotation is necessary meaning that peas can be cultivated only once every six years on the same location (Helsper et al., 2006).

Field beans are susceptible for soil-borne pathogens, like Fusarium spp. and Pythium spp. The white-flowering varieties are in general more susceptible than the coloured-flowering ones. Crop rotation is very important to reduce pressure by soil-born pathogens. (Helsper et al., 2006).

Lupine is sensitive to several plant pests, such as fungal pathogens (e.g. Colletotrichum gloeosporiodes) which causes anthracnosis and may result in a yield loss up to 50% (Helsper et al., 2006). Humid and warm weather enhances the disease spread. Some lupine species are susceptible to Fusarium. Therefore, a crop rotation schedule of one to four (maximally one lupine crop per four consecutive years) is required (Helsper et al., 2006).

Lucerne can fixate nitrogen due to a symbiotic relationship with bacteria. In the last decades lucerne with a better disease resistance has developed but it is still very sensitive to several bacterial, fungal, viral and parasitic diseases (Van Krimpen et al., 2013). Lucerne contains a lot of water and generally a drying process is required prior to storage.

Grassland is mainly used for grazing of ruminants and horses and the production of silage and hay. Van Krimpen et al. (2013) concluded that grass can to some extend contribute to the protein supply of pigs. This, however, would need a drying step. This also applies to sugar beet leaves or other green leaves.

Quinoa grows on all soil types, provided that they are reasonably permeable. Heavy and easily compactible soils are less appropriate. Quinoa is related to beet and should, therefore, not be preceded by beet as a crop. Quinoa is resistant towards nematodes and rhizomania (Helsper et al., 2006).

Algea are regarded as a very promising source for the production of biofuels, feed and food (Wijffels and Barbosa, 2010). The best cultivation conditions and selection of the best performing algae species and strains depending on their application are investigated. Bulk scale production of microalgae will take 10-15 years (Wijffels and Barbosa, 2010). Serious problems to overcome include cultivation/fermentation (increasing yield per hectare), harvesting (because of their small size) and biorefining (open up the firm cellulosic cell wall).

Legislation and costs

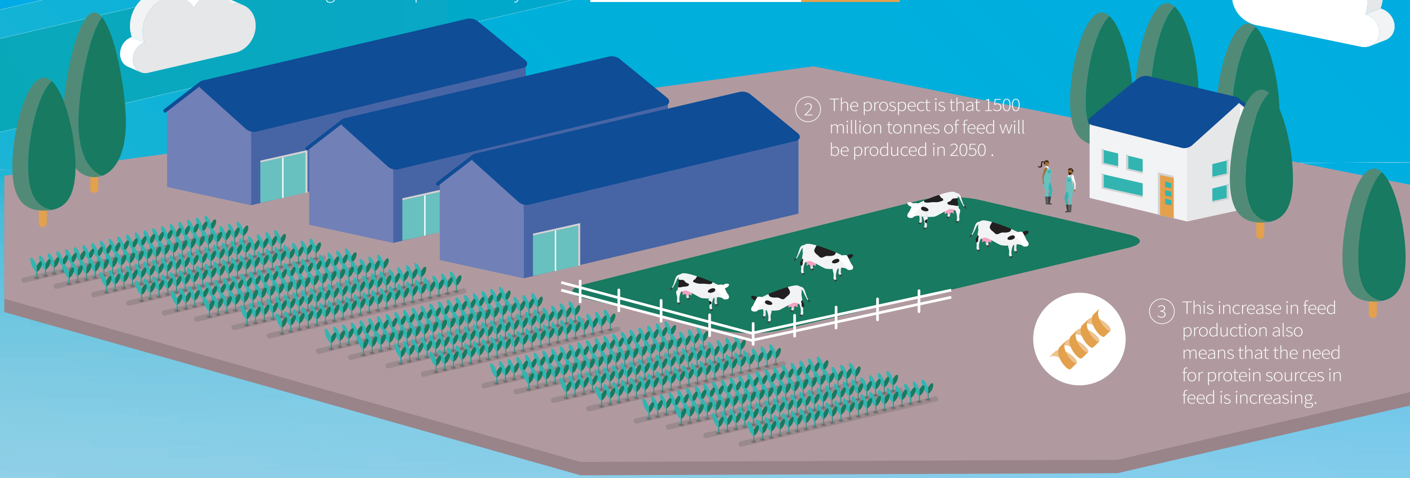
High production costs might be a bottleneck for cultivation of micro-algae solely for protein production (Van Krimpen et al., 2013). To compete with soybean meal as a protein source, the cost of algae have to be reduced. This requires the development of innovative, productive algae systems with reduced installation costs and low energy costs (Spruijt et al., 2014).

Main bottlenecks for use of insects in the near future are in the area of legislation and the achievement of a low cost price by an automation of the production process (Veldkamp et al., 2012). It is forbidden to use animal proteins in feed.

Plant-based proteins could meet rising demand for animal feed

(1) The European feed production increased by 23% from 2005 till 2012, the global feed production by 47%.

Europe	+23%
Global	+47%



Sources of protein in compound feed

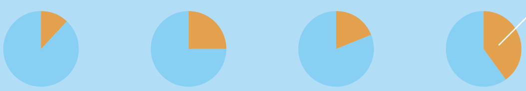
- Oil seeds
- Grain legumes
- Insects
- Leafs
- Forage legumes and leaves
- Aquatic biomass
- Cereals and pseudo-cereals
- Single cell proteins

European production and yield

Comparing the three most produced feed crops in Europe, grass, rapeseed and lucerne, with soybean.

	grass	rapeseed	lucerne	soybean
Area production in Europe (x 1.000 ha)	238,000	8,770	7,120	2,740
Yield in Europe (x 1.000 t)	2,380,000	23,080	78,320	4,790

Protein content (%)



Soy beans have the highest nutritional value, but relative low yield in Europe.

Bottlenecks

Anti-nutritional factors (ANF)
ANFs are compounds in protein containing crops which can negatively affect the nutritive value.

Cultivation
Crops like soy beans have a relative low yield in Europe, making them less attractive.

Pathogens
Peas are very sensitive to pathogens and pests.

Processing
Grassland can contribute to protein supply of pigs, but production needs an additional drying step.

Innovation
Algae show promise as biomass source, but serious problems need to be overcome first, including: cultivation, harvesting and downstream processing.

Costs
High production costs might be a bottleneck for cultivation of micro-algae and their use in animal feed.

Legislation
Legislation may be a bottleneck in production of insect protein. For the time being, it is forbidden to use animal proteins in feed.

Plant-based proteins could provide new and sustainable feed products for animals to meet the growing demand for protein

5. Proteins in biobased applications

Next to uses of proteins in the food and feed sector, proteins can also be used in more technical biobased applications. Protein rich residues produced by the agro sector and industry, and new protein sources, potentially can be valorised as binders in coatings and adhesives, as surface active agents and as green chemicals.

PROTEINS USED IN BIO-BASED APPLICATIIONS – REQUIREMENTS

In the past proteins were commonly used in non-food applications. Henry Ford was an important early pioneer in soy protein utilization, applying these sources to improve his automobiles. Products such as plastics were developed. Several end-applications have been based on casein and soy protein. In the paper industry, proteins have been used as sizing agents, binders, and adhesives. Glue, derived from collagen, has been used for bonding paper and as an adhesive in paper coatings. Plywood adhesives based on soy protein have been developed. Hydrolysates from keratin, gelatine, and wheat gluten have been used in cosmetics, as surfactants in shampoos.

When developing technical applications based on industrial proteins, specific functional properties and requirements should be considered depending on the target application. Important functionalities are:

- Water solubility and processing in water or in the melt
- Emulsifying and foaming properties, i.e. surface active properties
- Gelling properties
- Film forming properties
- Adhesion properties
- Barrier properties for oxygen and carbon dioxide
- Resistance against solvents

To replace synthetic materials by renewable proteins, there are some additional requirements needed for industrial implementation, such as that the materials must be processable on machinery used for the synthetic materials and their economic feasibility.

From a general point of view, proteins can be used in the following technical applications:

- Adhesives
- Coatings
- Surfactants
- Bioplastics

The type of functionality that is required depends on the end application. Examples are adhesion and bond strength for adhesives, resistance against water for coatings, and strength for plastic materials. Table 15. gives an overview of a number of technical applications of proteins, the requirements, and respective routes to obtain good product performance.

Table 15. *Technical applications of industrial proteins*

Product	Protein	Example	Functionality
Adhesive	casein, wheat gluten, soy protein, gelatine	water based hot melt	processing tack bond strength
Coating	soy protein, casein, zein	paint ink paper/packaging coating	film forming properties strength water resistance
Surfactants	keratin, wheat gluten	emulsifier detergent wetting agent	surface tension stabilisation of interface
Plastic	soy protein	packaging	melt strength tensile strength water resistance

During the last decades, there has been an increasing demand from consumers and industries to replace synthetic polymers with polymers from renewable resources. In addition to this, new streams of biomass that contain significant amounts of proteins will become available in the near future due to more sustainable biomass use in biorefinery processes. In some cases, like Jatropha protein, that is not suitable for food consumption, these streams have potential possibilities to be used in the non-food sector.

SUSTAINABLE PROTEIN SUPPLY

As a consequence of the increasing world population, the necessity for a sustainable protein supply is increasing. The search for new protein sources, such as: micro-algae, macro-algae (seaweed), duck weed, grasses, leaves, insects and crustacean waste (e.g. lobster, crab), and the valorisation of protein rich residues from the ethanol production from wheat and corn, meat producing industry, fish industry and press cakes of soy, rapeseed and sunflower are important issues.

The most important outlet for proteins is the food and feed market, however, not all proteins can be used in food and feed due to quality and regulatory limitations. These proteins potentially can be applied in technical applications, such as: coating systems, adhesives, surface active agents and as so-called 'green chemicals'. The expectation is that the market for green chemicals will increase over the next few years. Currently, European UNIs/RTOs and industries/SMEs are collaborating with national/international GOs/NGOs to develop strategies and methodologies for the optimal sustainable use of protein-rich biomass sources for Food, Feed and Non-food applications.

CURRENT BIO-BASED APPLICATIONS

About 0.3 Mtonne of proteins is used in glues and other applications (see Table 16.). These proteins are mainly from animal origin, like gelatines or caseins (Bos, Elbersen et al. 2010). An important current application area for proteins is glues; these are rather long-standing applications, but they are in continuous competition with glues from petrochemical origin, since these offer in many applications better performance for a lower price. Proteins thus still find limited technical applications in the non-feed, non-food industrial markets.

ASPECTS FOR FUTURE BIO-BASED APPLICATIONS

In certain niche markets, such as casein as labelling adhesive or gelatine as book binding glue, proteins can be used as a biopolymer. The functionality of the intact molecular structure is being used. Probably, this market will stay, and might even expand somehow. Expansion is being restricted due to the intrinsic properties of the proteins. For example, the use of proteins as a biopolymer in coating material is limited to a number of applications because of the water sensitivity of the protein compared to synthetic polymers. Adjusting the properties by means of physical or chemical modifications is only possible to a certain extent. Water sensitivity, for example, can be improved by cross-linking or attaching hydrophobic moieties to the protein back bone. Another restriction in using the whole protein structure is the fact that the history, the process condition during protein isolation, has a dominant effect on the functional properties. For example, the functionality of the proteins that are present in the press cakes of oil seeds, such as soy or rapeseed cake, are lost to a high extent. The reason is that the extraction procedure has been optimised towards oil yields. During this extraction process high temperatures and the use of hexane cause severe denaturation of the proteins.

Table 16. *Non-food applications of proteins (partly, according to Eurostat)*

Product	Remark	Amount [ktonne]
Wheat gluten	About 10% of the amount of wheat gluten in e.g. pharmaceutical pills, adhesives and surfactants	45
Peptones and derivatives, other protein substances and derivatives, hide powder, keratin		34
Casein adhesives		40
Gelatine		40
Bone glue	Except casein adhesive	15

An alternative way of using protein is as hydrolysate. An advantage is that the three dimensional structure of a hydrolysed protein is not present anymore and therefore it can also be assumed that the nature of the protein, denatured or not, has less impact on the functionality of the protein hydrolysate. Another aspect is that hydrolysis usually is needed to obtain protein structures with surface active (such as emulsifying) properties. Therefore, protein hydrolysates can, for example, be used in cosmetic products such as shampoos and creams, but also in coating systems. Products based on gelatine hydrolysates, hydrophobised with fatty acids have been produced on commercial scale. Surfactants based on proteins can also be used as an adjuvant in crop protection or as bio-flocculants.

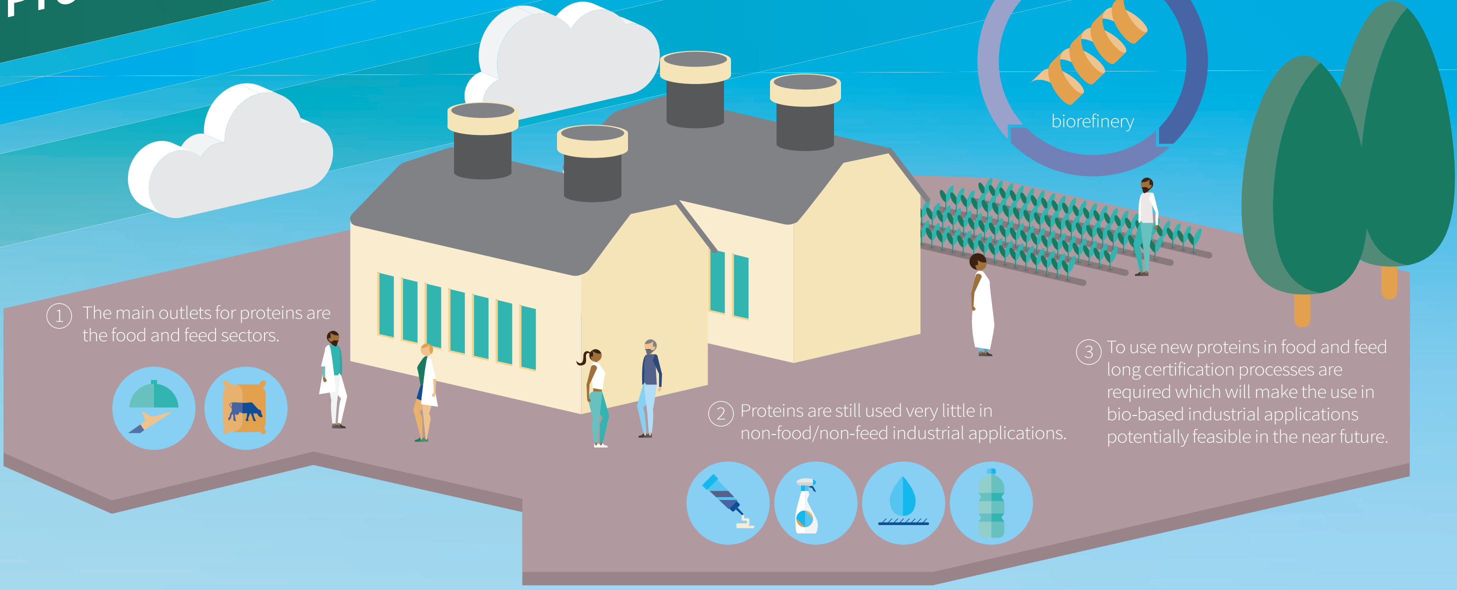
In addition to protein hydrolysis, the exploitation of proteins can also be increased by extracting specific protein fractions from the total protein content. By doing so, protein structures with well-defined properties can be produced. More research, however, is still required to find relationships between the structure and functionality needed for certain end-applications.

"New" protein sources like grass, beet leaves, micro and macro algae, press cakes, and residues of the meat and fish industry have potential for technical uses. These types of protein-rich biomass are much cheaper than the isolated proteins that are available on the market. A lot of research is carried out with respect to the use in a biorefinery of these various biomasses. Many hurdles, however, still have to be solved on the separation, isolation and purification techniques.

BIOREFINING AND PROTEIN BASED RESEARCH TOPICS

Refining of protein-rich biomass sources, like conventional and new crops, agro residues, process residues and post-consumer residues, potentially offers the opportunity to efficiently process the available biomass potential into a portfolio of marketable biobased products (incl. proteins for food, feed and technical applications) and bioenergy. However, major technical and non-technical barriers still have to be solved before this promising potential can fully commercially deployed. Major technical R&D topics are mild fractionation, separation/isolation and purification of protein-rich sources into intermediates or products with required functionalities for further downstream processing or selling; non-technical deployment barriers are mainly in the field of governmental regulations and public perception and acceptance.

Proteins in bio-based applications

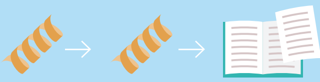


Current protein uses

Proteins have been used for a long time in the biobased industry.

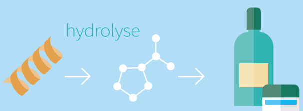
product	protein	example	functionality
adhesive	casein, wheat gluten, soy protein, gelatine	water based hot melt	processing tack bond strength
coating	soy protein, casein, zein	paint ink paper/packaging coating	film forming properties strength water resistance
surfactants	keratin, wheat gluten	emulsifier detergent wetting agent	surface tension stabilisation of interface
plastic	soy protein	packaging	melt strength tensile strength water resistance

New uses for proteins



Intact 3D protein structure used

Using casein proteins as a biopolymer in labelling adhesive or book binding glue.



3D Protein structure not present anymore

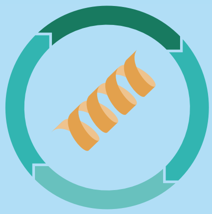
Using proteins as hydrolysate used in shampoos and creams, and also in coating systems.

A more sustainable protein supply chain

As a consequence of the increasing world population, the necessity for a sustainable protein supply is increasing.

1. New protein sources: Micro- and macroalgae; Grasses and leaves; Insects; Crustacean waste
2. Valorisation of protein rich side streams from: Ethanol production; Meat industry; Fish industry; Press cakes of oil seeds and soy
3. Technical applications: Coating systems; Adhesives; Surface active agents; 'Green' chemicals

Increase protein production with biorefinery



Co-production of proteins with other biobased products and bioenergy using efficient biorefinery processes is the optimal way for sustainable biomass use in the future Circular BioEconomy.

Protein use in technical biobased applications can be very promising for using "new" protein sources to prevent the long certification processes needed for used in the food/feed sectors

6. Biorefining of protein containing biomass

OIL CROPS

The demand for vegetable oils has been rising for many years. Worldwide, palm oil has the highest production volumes, closely followed by soybean oil. In Europe the four most important oil crops are: rapeseed, sunflower seed, soybean and olives.

Recovery of oil from oilseeds is mainly done with screw presses. In order to increase the oil yield the seeds can be pre-treated by preconditioning. Often the pressing stage is followed by an extraction step usually with hexane to recover the residual oil from the press cakes. The oil production using rapeseed and sunflower seeds results in waste streams that still contain valuable compounds like proteins.

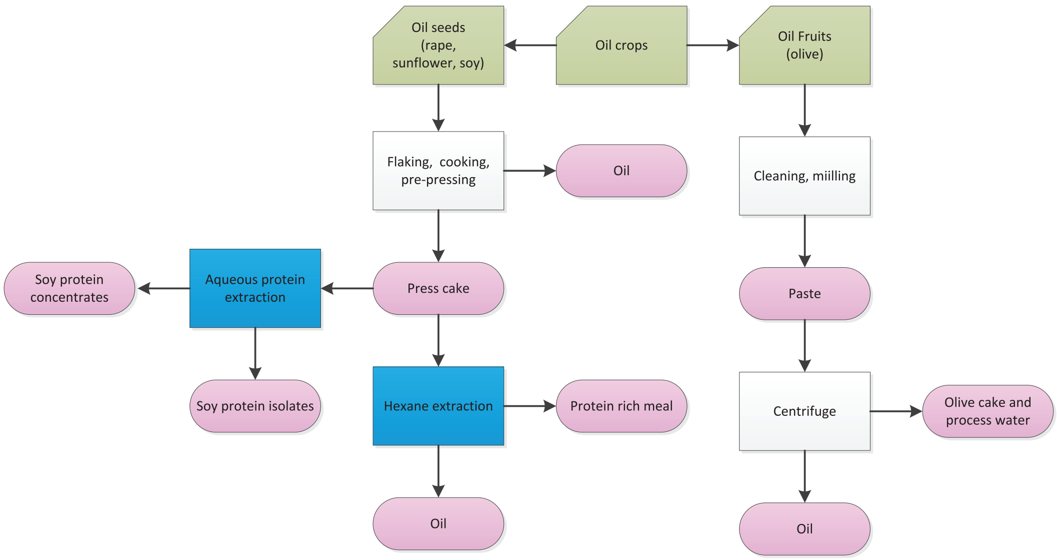


***Figure 13**. Conventional oil crop refinery [Wageningen UR]*

Rapeseed provides oil (circa 40% by weight) and a residue, the meal (circa 60% by weight). The rapeseed meal consists mainly of proteins (40-45 wt%), fibres and secondary metabolites (e.g. glucosinolates, phenolic compounds). The animal feed market is the main outlet for rapeseed meal and its constituents.

The major producing countries for sunflower seeds are: Argentina, EU countries, Russian Federation, and other Eastern European countries. Oil and proteins are the main components of the sunflower seed. Sunflower kernels consist of about 20-40 wt% proteins. Sunflower meal is obtained as a by-product of the oil extraction process and has a high protein content; about 40-60 wt% depending on the extraction method. This high protein content makes sunflower meal an attractive source for the isolation of proteins. As compared to proteins from legumes and other oilseeds, sunflower proteins have no anti-nutritional components. Sunflower seeds, however, have a high content of phenolic compounds. They attribute to the dark colour of protein products. The interactions of phenolic compounds with proteins can affect the protein properties in several ways, such as reducing protein digestibility and functionality, prolonging or shortening its shelf life and storage stability.

On average, soybeans contain about 40 wt% protein and 20 wt% oil. So protein is the component present in the highest amount; soybeans can therefore be considered more as protein rather than oil source.

Olive oil is mainly being produced in Europe, and the main producing countries are: Spain, Italy, Greece, Portugal, Slovenia and Croatia. Olive oil is specifically used in cooking. Oil is isolated from the olive by the use of centrifuges (two phases or three phases). The process generates by-products like olive leaves, olive press cake and oil mill waste water. Apart from these major oil crops the spectrum of vegetable oils is much broader, for example for niche products like: hemp seed oil, cotton seed oil, linseed oil and Jatropha seed oil.

Table 17. *Total worldwide and European production of protein-rich press cakes [Million tonnes, source OilWorld]*

Meal	World production	European production
Soy meal	190.5	10.4
Rapeseed meal	38.0	13.5
Sunflower meal	17.5	4.1
Palm kernel meal	7.9	0

The press cakes in general contain about 35-50 wt% protein. Due to the oil extraction process by hexane, the proteins are denaturated to a large extent, resulting in meals with a high content of insoluble proteins. Therefore, the main outlet for these press cakes is in animal feed.

To improve the functionality, and thereby the added value of the protein fraction, adaptations in the oil extraction factory are necessary. Although there are good reasons to investigate the possible adaptations, it will be difficult to implement changes, since these factories are fully optimized towards maximum oil productivity and not from a biorefinery point.

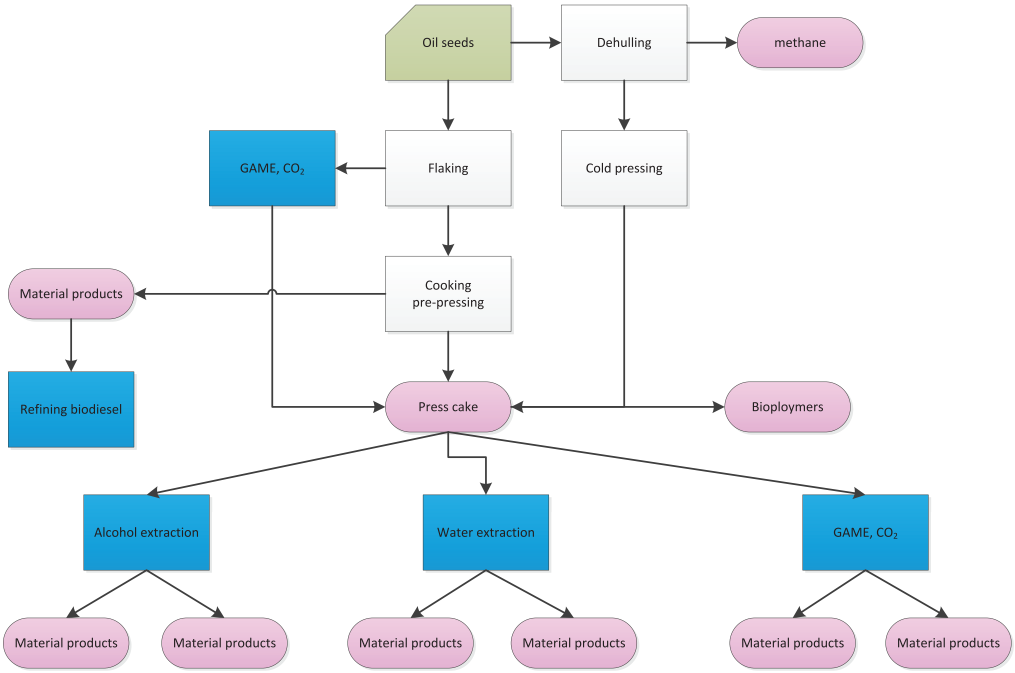


***Figure 14**. Advanced oil crop refinery [Wageningen UR]*

STARCH CROPS

Different agricultural crops with a high content of starch are being cultivated. Examples are: potatoes, grains/cereals (e.g. wheat, rye, maize) and legumes (e.g. (chick)pea, white/brown beans). Apart from being used as such, mainly for human food, a number of these crops are also being used as starting material for the production of starch. An overview of the processing of the different starch crops is depicted in Figure 15.

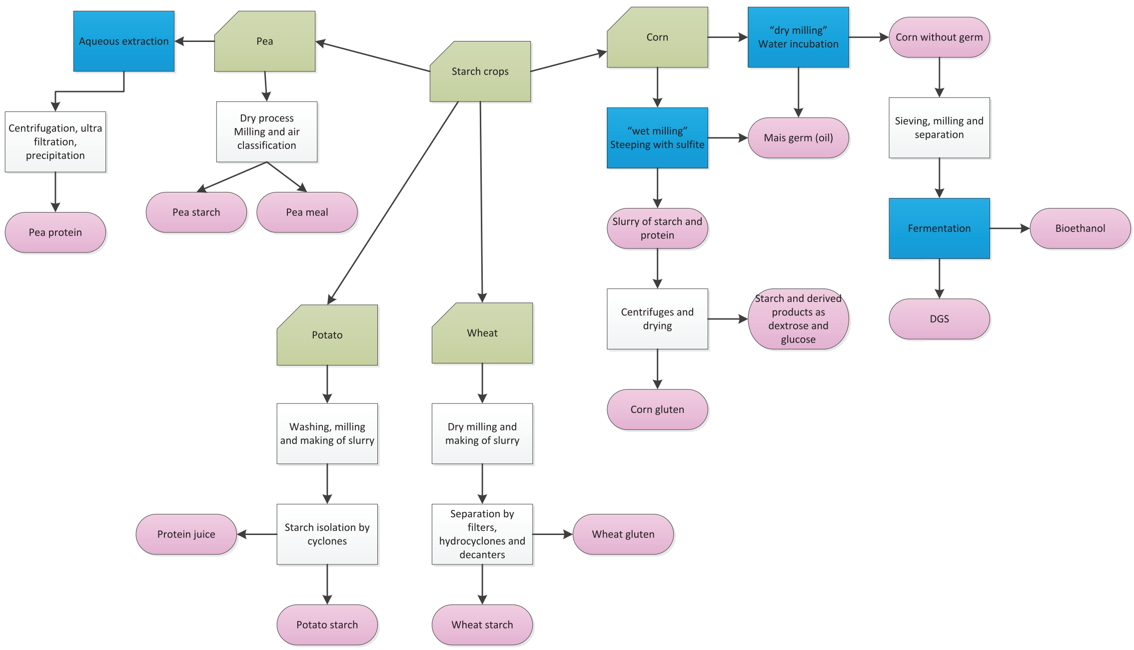


***Figure 15**. Conventional starch crop refineries [Wageningen UR]*

In addition to the production of proteins, nowadays, these crops are also being used as starting material for the production of bio-ethanol. In Figure 15 this is shown for corn, but is can also be applied on wheat or another starch containing crop.

The standard potato processing industry results in a protein product with very low functionality. Solanic, an AVEBE group company, has developed a biorefinery process, using chromatography, by which the isolated proteins maintain their functional properties. These minimally processed potato proteins have excellent water binding, fat binding and foaming properties and, since the manufacturing process has received food safety certification, Solanic achieved a GRAS Notice status, which means that the process derived proteins can be used in several food applications.

The Solanic potato refinery process is schematically shown in Figure 16. The raw potatoes are washed and rasped to produce a potato juice, which is mechanically separated from the potato solids. Subsequently, fibres and any remaining undesirable fine particulates are removed. The potato proteins are isolated, and purified from the juice fractions, using different chromatographic methods, resulting in a high and low molecular weight fraction. Specifically, the high molecular weight fraction is above 35 kDa and the low molecular weight fraction between 4 and 35 kDa. These fractions are concentrated by ultrafiltration and conditioned by mild temperature treatment (temperature ranges between 20 to 25°C). The final concentrates are spray dried or stored as stable concentrates. UV treatment is carried out to control and reduce microbial infections.

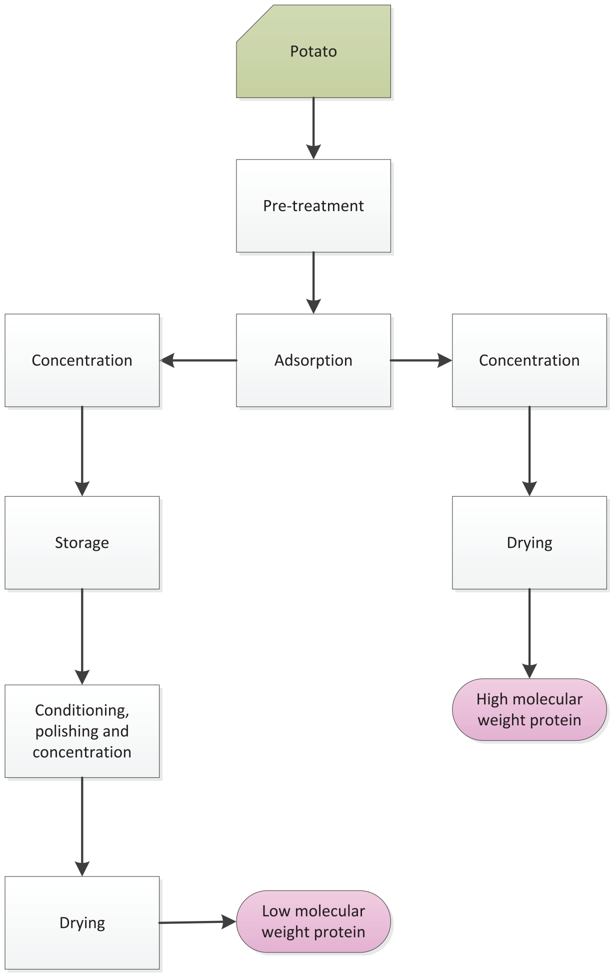


Figure 16*. Advanced potato refinery process (Solanic) producing proteins for food applications [Wageningen UR]*

The basic biorefinery of corn is being carried out by the wet milling technology – see Figure 15. – yielding corn oil, corn fibres and corn starch. In the U.S. the corn starch fraction is being used as such (16%) or transferred into corn syrup (36%), dextrose (17%) or bioethanol (31%). Wet milling of corn also generates corn gluten meal. An important step in the wet milling is the so called steeping process, by which a sulphite solution is being used to soften the maize kernel. This process step has the drawback that the proteins present in the cakes have lost the majority of their functional properties and, therefore, can only be used as feed.

Byosis, a Dutch SME company, has developed an alternative biorefinery process for the production of bioethanol from maize, see Figure 17. The crucial step in this process is the gelling of the maize starch within the kernel. The great advantage of gelling the starch in its natural covering is the avoidance of a building up of the viscosity. After this gelling step, the starch is enzymatically converted in sugar moieties, and further on in the process bioethanol is produced. The lignocellulose parts of the maize grain are being processed to produce biogas. This biogas installation produces electricity and heat. The heat is being used for the distillation of the ethanol to reach a final concentration of 60%. Maize contains, besides lignocellulose and starch, a significant amount of proteins, mainly maize gluten and zein. Especially the zein is a protein with a high value of about 20 €/kg.

Since the Byosis process does not use sulphite, it is expected that the proteins could maintain their functional properties, and probably could be removed from the starch stream.

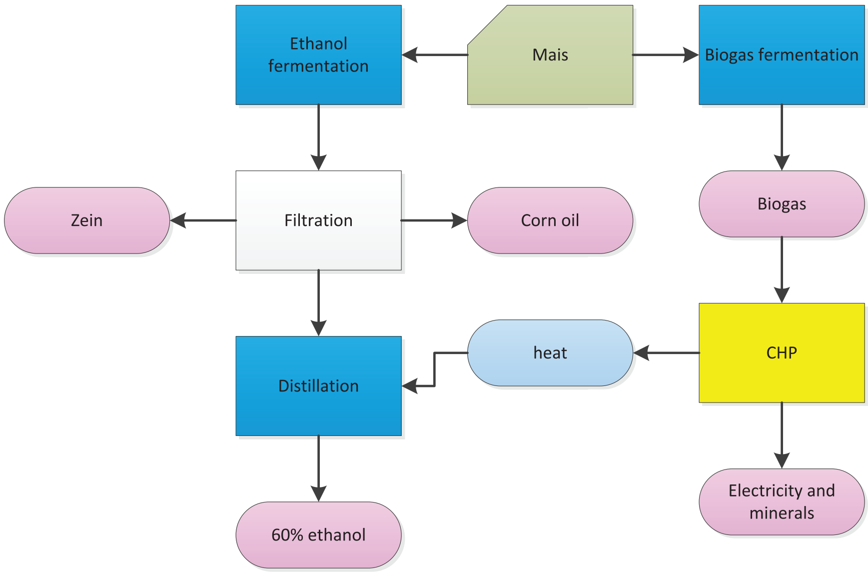


Figure 17. *Refinery process for zein, bioethanol, power and minerals from corn [Wageningen UR/Byosis]*

HERBACEOUS BIOMASS

Crops at which the proteins are embedded in a lignocellulose matrix are for instance grass, beet leaves and Lucerne. These herbaceous crops are primarily used as forage and a source of leafy vegetables.

Herbaceous biomass fractionation ("green biorefineries") is studied in many countries. The first step in the fractionation is usually a pressing step of the herbaceous crop dispersed in water, and a fibre rich press cake and a protein rich juice are obtained. In contrast to the press cakes of the oil crops, the press cakes of the herbaceous crops do not contain the protein fraction, but mainly starch and cellulose. The press cake can be used as feed pellets or as raw material for the production of chemicals. Upon heating the press juice the proteins coagulate and can be removed from the juice.

The first modern industrial process for leaf protein extraction was called the Rothamsted process. The procedure based on heat coagulation of green plant juice at 70°C, resulted in leaf protein concentrates with 60 wt% protein content. Later, procedures were developed based on a two-step heating of the green press juice resulting in products with different compositions. In general, the time between harvesting and processing should be as short as possible.

Grass and Lucerne juice have been used as feed as an alternative for e.g. fish protein or soybean meal. Leaf protein concentrates have fairly high contents of lysine and methionine.

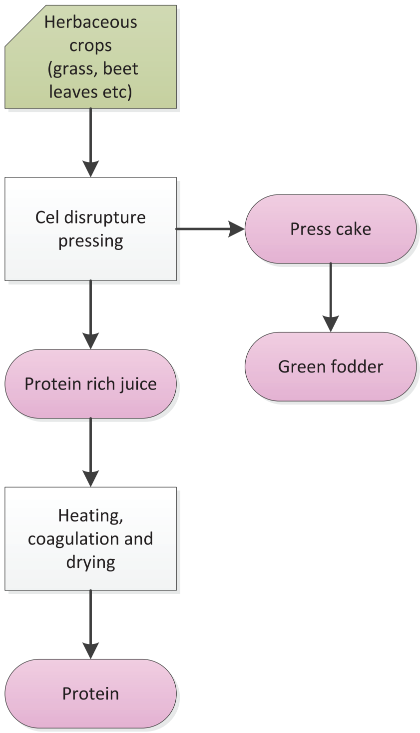


***Figure 18**. Herbaceous biomass refinery scheme [Wageningen UR]*

AQUATIC BIOMASS

Aquatic biomass – i.e. microalgae, macroalgae (seaweeds), aquatic plants (duckweed, etc.) – are high potential new biomass sources that can be used as raw materials in biorefinery processes – see Figure 19. – for the co-production of food/feed ingredients, biobased chemicals/materials, and bioenergy (fuels, power, heat).

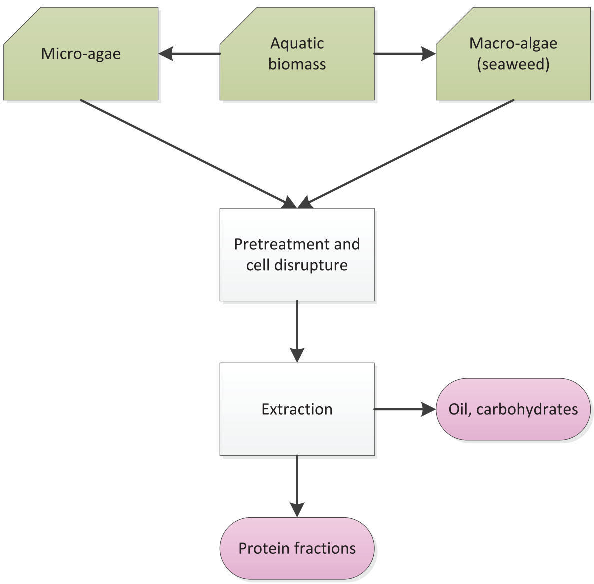


***Figure 19**. Aquatic biomass refinery scheme [Wageningen UR]*

Research on microalgae started about 10 to 20 years ago to find new sources for the production of oil. Research activities until recently were mainly focussed on the cultivation and harvest of different species, such as Chlorella and Tetraselmis. Microalgae contain, besides oils, carbohydrates and about 30-60 wt% proteins, and therefore research activities are nowadays directed to develop a process to isolate the oils, the carbohydrates and the proteins. These valuable components are located in the algal body, and are surrounded by the cell wall. Therefore, to obtain the desired components the cell wall has to be perforated or partly "destroyed". Since the value of the proteins that can be isolated is dependent on the functional properties, mainly mild pre-treatment and isolation processes are of interest. Finally, after the extraction and production of proteins, hydrolysated (peptides) and/or amino acids, methods are required that isolate and purify the materials. Protein hydrolysates are often complex mixtures, and therefore more advanced separation techniques, such as ion-exchange (IE), will be required. IE is effective in the separation of complex mixtures of amino acids, however, for large scale applications costs are anticipated to be high, due to elution times and the need for regeneration. Other purification techniques, like chromatography and crystallisation, might also potentially be used.

A large number of different seaweeds exist. Depending on the species, seaweed can have proteins contents between 5 and 40 wt%, and therefore are an interesting source for protein isolation. At this moment the main focus for the production and use of seaweed is as food and hydrocolloids, such as: alginate, agar and carrageenan. However, new developments take place in which seaweed is being cultivated for the production of sugars, producing ethanol after a fermentation process. Especially, for this last application it is shown that no commercial process can be developed only based on the production of sugars for alcohols production. To make this processes economically viable, it is important to extract and valorise the proteins. Most of the native proteins are water soluble at a certain pH. Therefore, proteins can be isolated in acidic or alkaline solutions. In mixtures, where apart from proteins also other materials like fat or cellulose are present, extraction efficiency can be increased by the use of enzymes (cellulases, amylases, proteases). The use of acids, bases, or enzymes, however, can hydrolyse the protein material during the isolation process. This has to be taken into account for applications in which the molecular weight of the protein is important. In general, the isolation process has an effect on the chemical and physical properties of the proteins. Like for the isolation of proteins from microalgae, after pre-treatment and isolation, the proteins have to be purified and dried.

About forty different types of duckweed exist. About 7-40 wt% of the dry matter is protein, depending on the species and the growing medium. The main protein fraction is Rubisco. The amino acid composition is very similar to that of soy protein. The cells of duckweed can easily be opened in comparison to the cells of most of the micro and macro algae.

DRIED DISTILLERS GRAINS WITH SOLUBLES (DDGS)

Industrial processes can generate water streams that contain certain amounts of proteins. These streams can be very diverse, however, one example is Dried Distillers Grains with Solubles (DDGS).

The conversion of wheat or maize into bioethanol is an example of a process where also an aqueous protein containing stream is produced. Typically these first generation ethanol fermentations have high conversion rates, converting close to 95% of the glucose derived from the starch. This leaves about 30% and 45% of the corn and wheat feedstock, respectively, unfermented in the broth medium. Ethanol is separated from the broth medium by distillation columns. This process step reduces strongly the protein quality.

The fresh distillers grains are first subjected to a cyclone to separate the insoluble solids portion from the soluble and majority of the water content. The aqueous soluble stream is evaporated to remove a large portion of the water. Afterwards, the insoluble solids and solubles streams are recombined and dried down in a rotary drum drier to a final moisture content of less than 10 wt%, thus producing dried distillers grains with solubles (DDGS). After a great part of the carbohydrates have been converted to and recovered as ethanol, the biofuel residues will be left with a large protein content. However, it has to be considered that a significant amount in for example DDGS is yeast. Some of the residues are so high in protein content that they can be used directly as animal feed, and are marketed for their relative high protein content. These protein contents can be compared with pure corn gluten meal. A feasible option would be to remove and isolate the large protein and/or amino acid content in a pure form for feed applications.

BIOEFINERY FACT SHEETS (BFSs)

In the last triennium, IEA Bioenergy Task42 has developed a BFS-methodology to both describe biorefinery process key characteristics and present major full chain sustainability assessment results. Below two BFSs – made by Joanneum Research GmbH (AT) based on data-input from Task42 members – are presented showing the advantages on overall sustainability level of co-producing proteins with secondary energy carriers.

BFS1 – Two-platform (C6 sugar, DGS) biorefinery producing bioethanol, feed, amino acids, phosphate and electricity from starch crops

Part A: Biorefinery plant

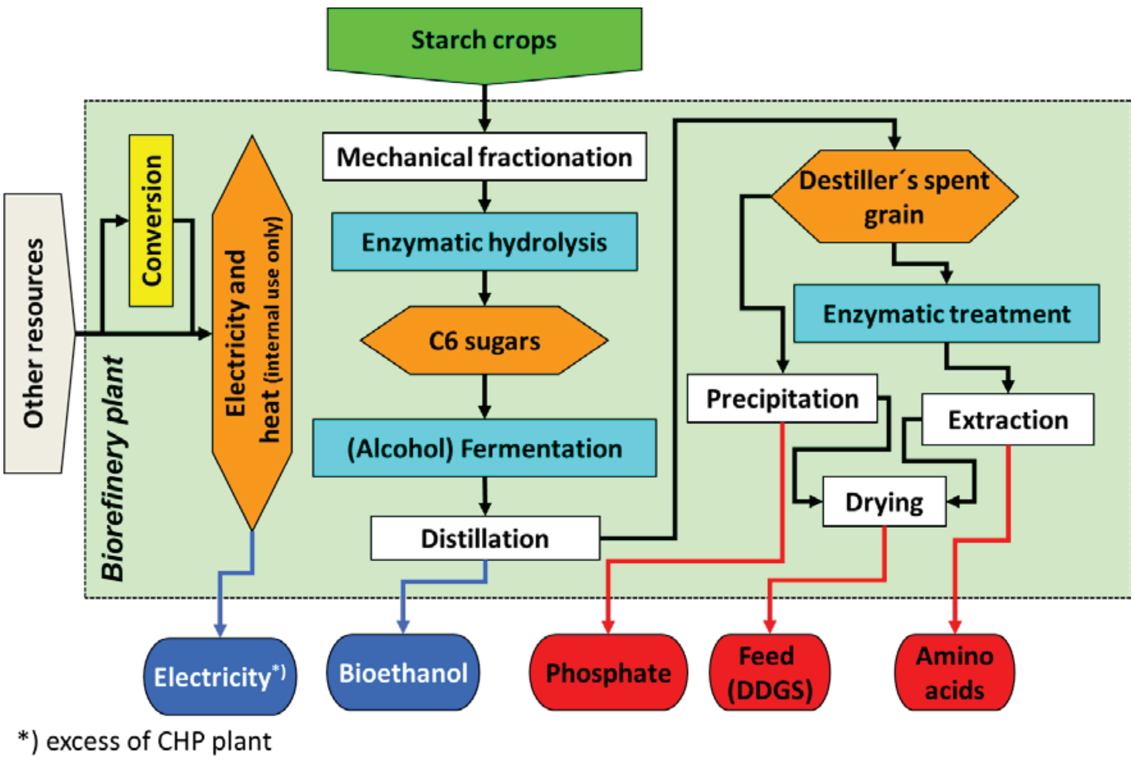


Figure 20. *Two-platform (C6 sugar, DGS) biorefinery producing bioethanol, feed, amino acids, phosphate and electricity from starch crops (IEA Bioenergy Task42)*

Table 18. *Key characteristics biorefinery plant*

2-platfrom (C6 sugar, DGS) biorefinery using starch crops for bioethanol, feed, amino acids, phosphate and electricity					
State of technology:	commercial, pilote: acids&phosphate production		Biorefinery Complexity Index (Platform/Feedstock/Product/Processes)		
Country:	EU 27				
Main data sources:	WUR, JOANNEUM RESEARCH				
Products			**Auxiliaries (external)**		
	bioethanol	400 [kt/a]		electricity	0.18 [PJ/a]
	DDGS	380 [kt/a]		heat	0.00 [PJ/a]
	amino acids	38 [kt/a]		energy carriers	8.5 [PJ/a]
	phosphate	4 [kt/a]			
	electricity	0.86 [PJ/a]			
Feedstock	[kt/a]	**water [%]**	**Costs**		
corn	1,268	15.0%		investment costs	400 [Mio €]
				feedstock costs	220 [€/t]
				number of employees	40 [#]
Efficiencies				**mass**	**energy**
		input to products		65%	65%
		input to transportation biofuel		32%	38%

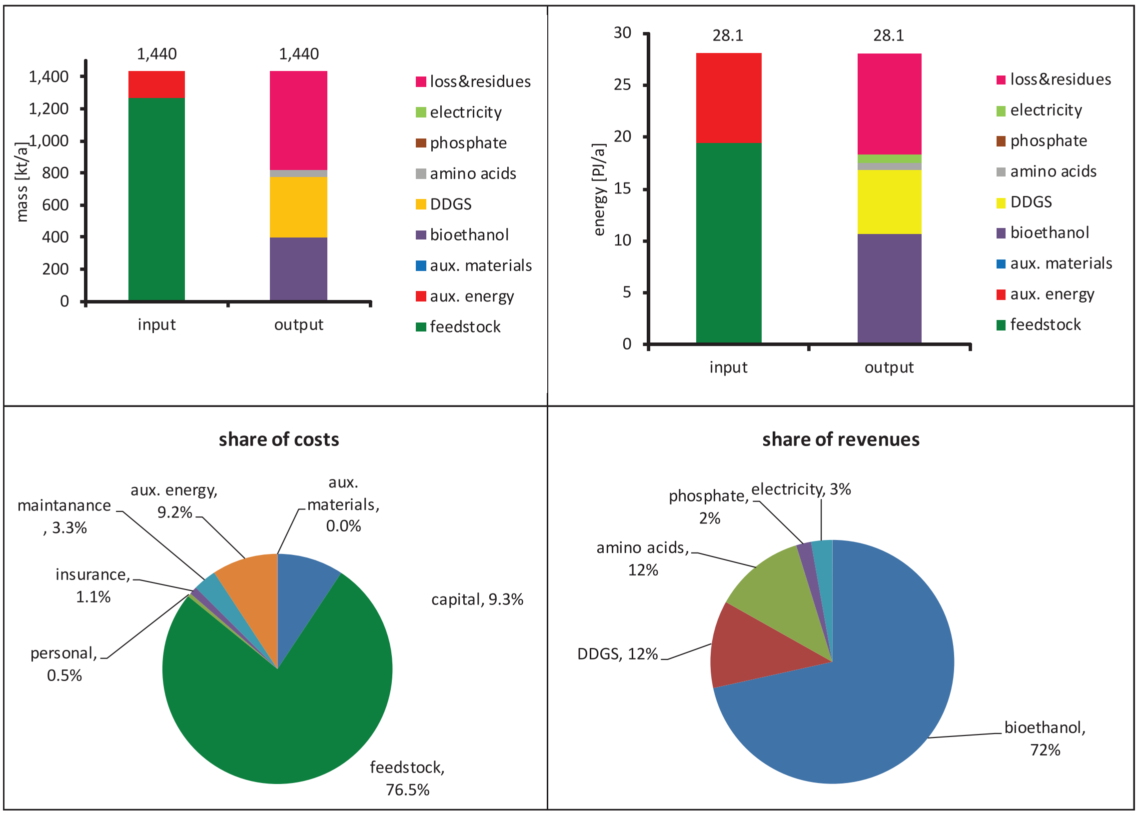


Figure 21. *Annual mass and energy balance of biorefinery plant; Share of costs and revenues of biorefinery plant*

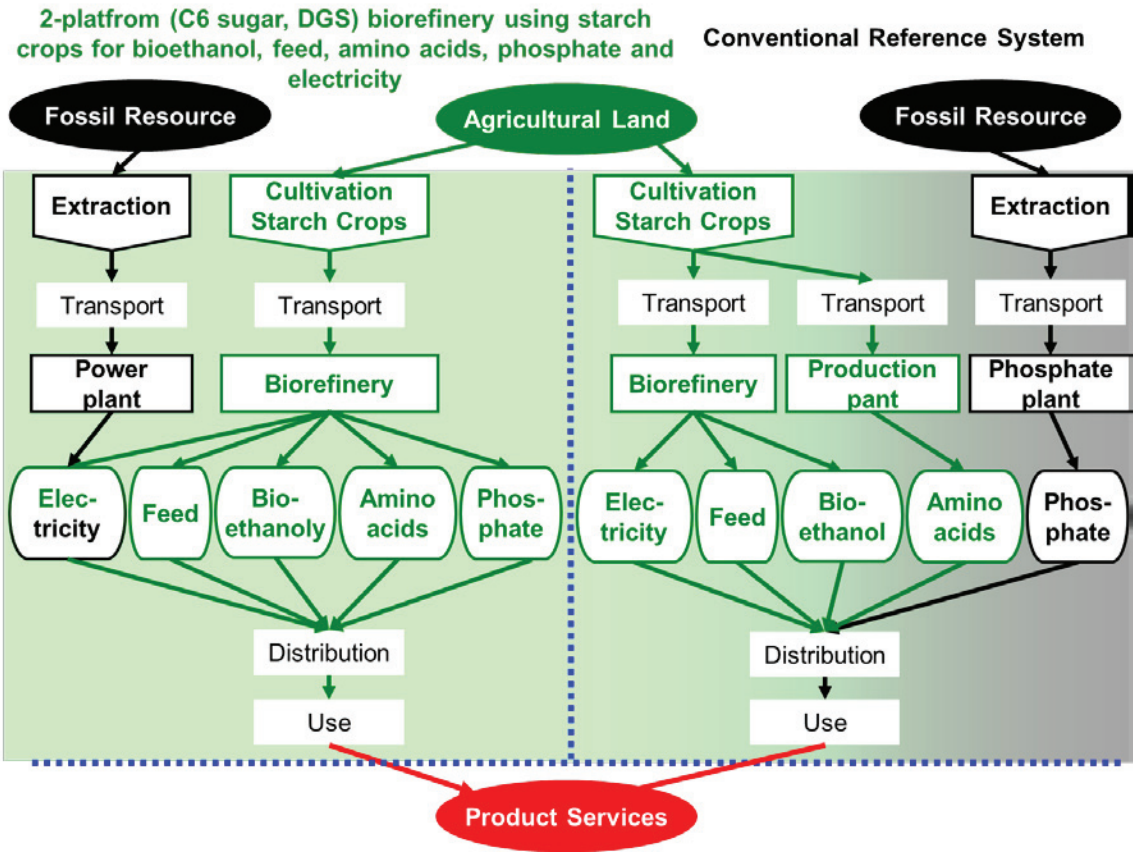


Figure 22. *Comparison biorefinery with conventional reference-system on full value chain (incl. "end of life treatment")*

Table 19. *Key characteristics biorefinery value chain*

Whole value chain			
Greenhouse gas emissions		**range**	
biorefinery	997	(930 to 1150)	[kt CO_2-eq/a]
reference system	1107	(1000 to 1300)	[kt CO_2-eq/a]
saving	-10%	(-9% to -11%)	[%]
Cumulated energy demand			
fossil			
biorefinery	13.2	(12 to 15)	[PJ/a]
reference system	14.2	(13 to 16)	[PJ/a]
saving	-7%	(-6% to -8%)	[%]
total			
biorefinery	33.6	(31 to 39)	[PJ/a]
reference system	35.8	(33 to 41)	[PJ/a]
change	-6%	(-6% to -7%)	[%]
Agricultural area demand			
feedstock	326,000	(304000 to 37500	[ha/a]
Costs			
annual costs	364	(340 to 420)	[Mio €/a]
specific costs	443	(410 to 510)	[€/t]
Revenues			
annual revenues	426	(400 to 490)	[Mio €/a]
specific revenues	518	(480 to 600)	[€/t]

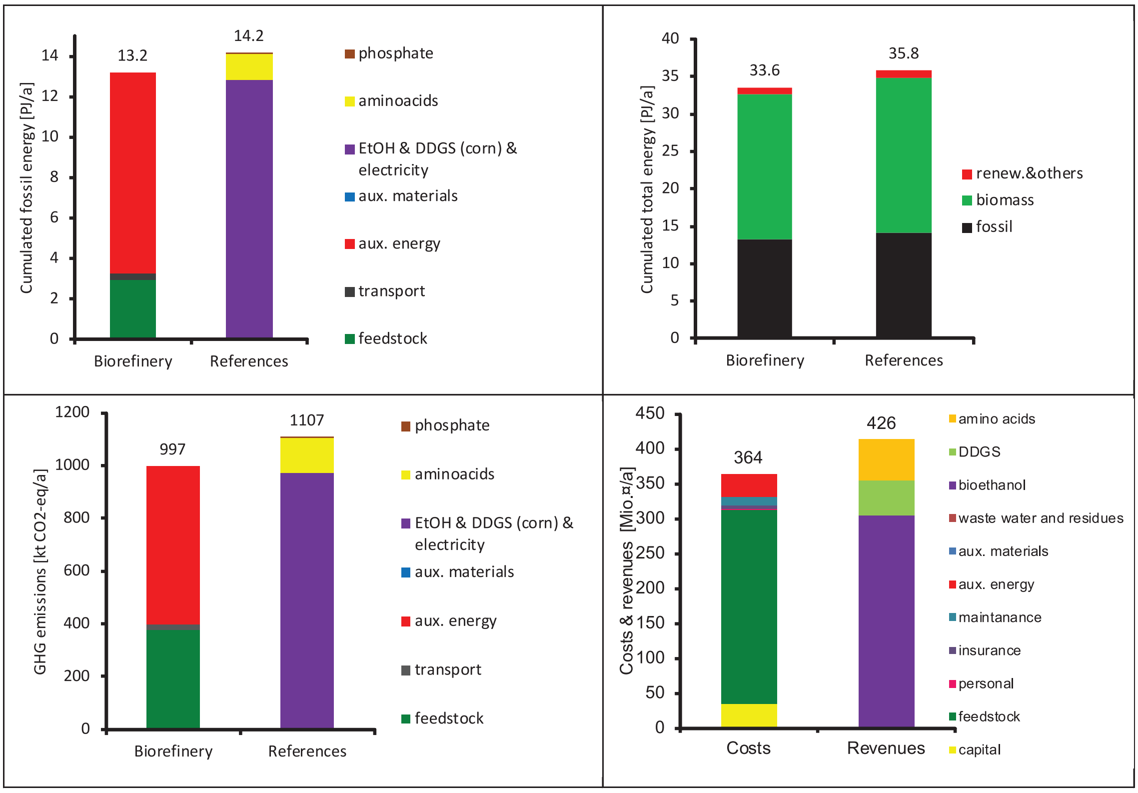


Figure 23. *Annual GHG emission, cumulated primary energy demand, cost and revenues of the full value chain*

BFS2 – Two-platform (biogas, electricity & heat) biorefinery producing protein, fertilizer, electricity from pasture & verge grass

Part A: Biorefinery plant

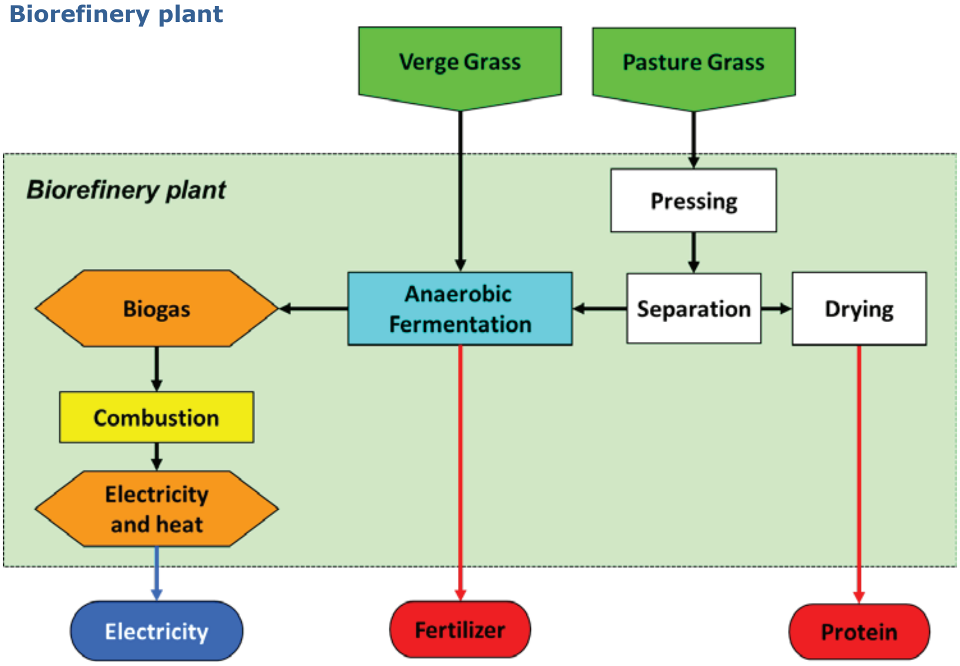


Figure 24. Two-platform (biogas, electricity & heat) biorefinery producing protein, fertilizer, electricity from pasture & verge grass

Table 20. Key characteristics biorefinery plant

2-platform (biogas, electricity&heat) biorefinery using pasture&verge grass for protein, fertilizer, electricity					
State of technology:	commercial, pilote: separation of protein		Biorefinery Complexity Index (Platform/Feedstock/Product/Processes)		
Country:	EU 27				
Main data sources:	WUR, JOANNEUM RESEARCH				
Products			**Auxiliaries (external)**		
fertilizer	66 [kt/a]		electricity	0.006 [PJ/a]	
protein	4 [kt/a]		heat	0.00 [PJ/a]	
electricity	0.086 [PJ/a]				
Feedstock	**[kt/a]**	**water [%]**	**Costs**		
pasture grass	78	65.0%	investment costs	10.0 [Mio €]	
verge grass	10	65.0%	feedstock costs	19 [€/t]	
			number of employees	4 [#]	
Efficiencies				**mass**	**energy**
			input to products	78%	34%
			input to transportation biofuel	0%	0%

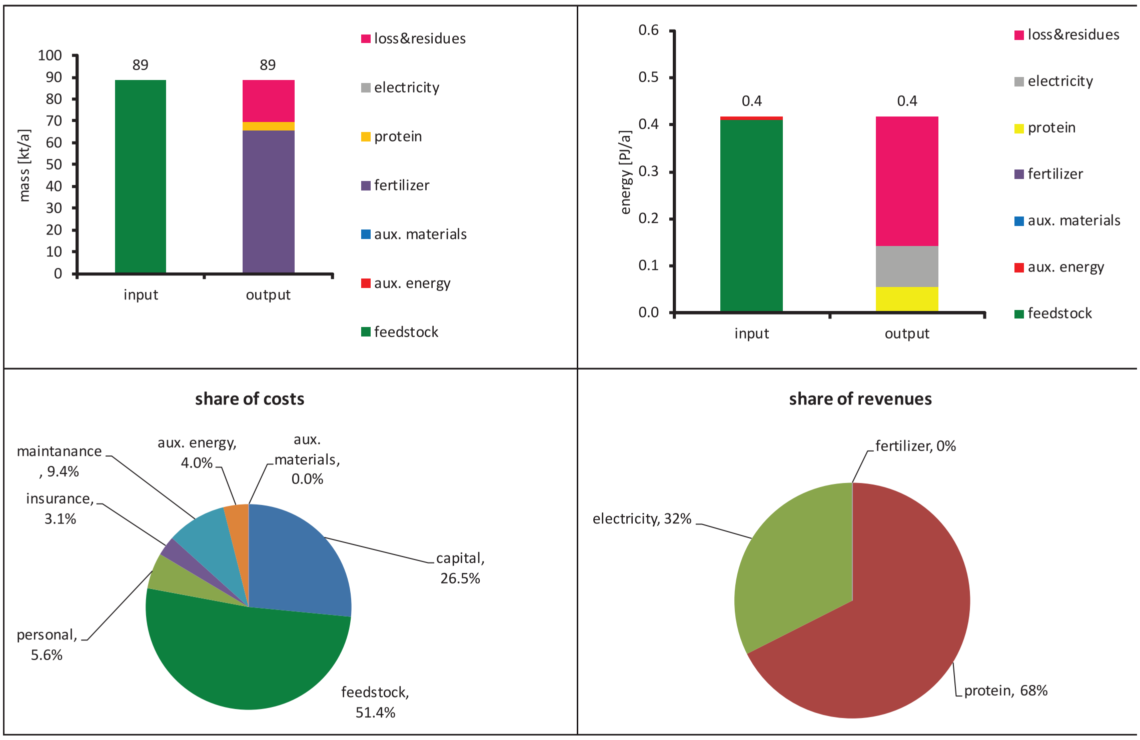


Figure 25. *Annual mass and energy balance of biorefinery plant; Share of costs and revenues of biorefinery plant*

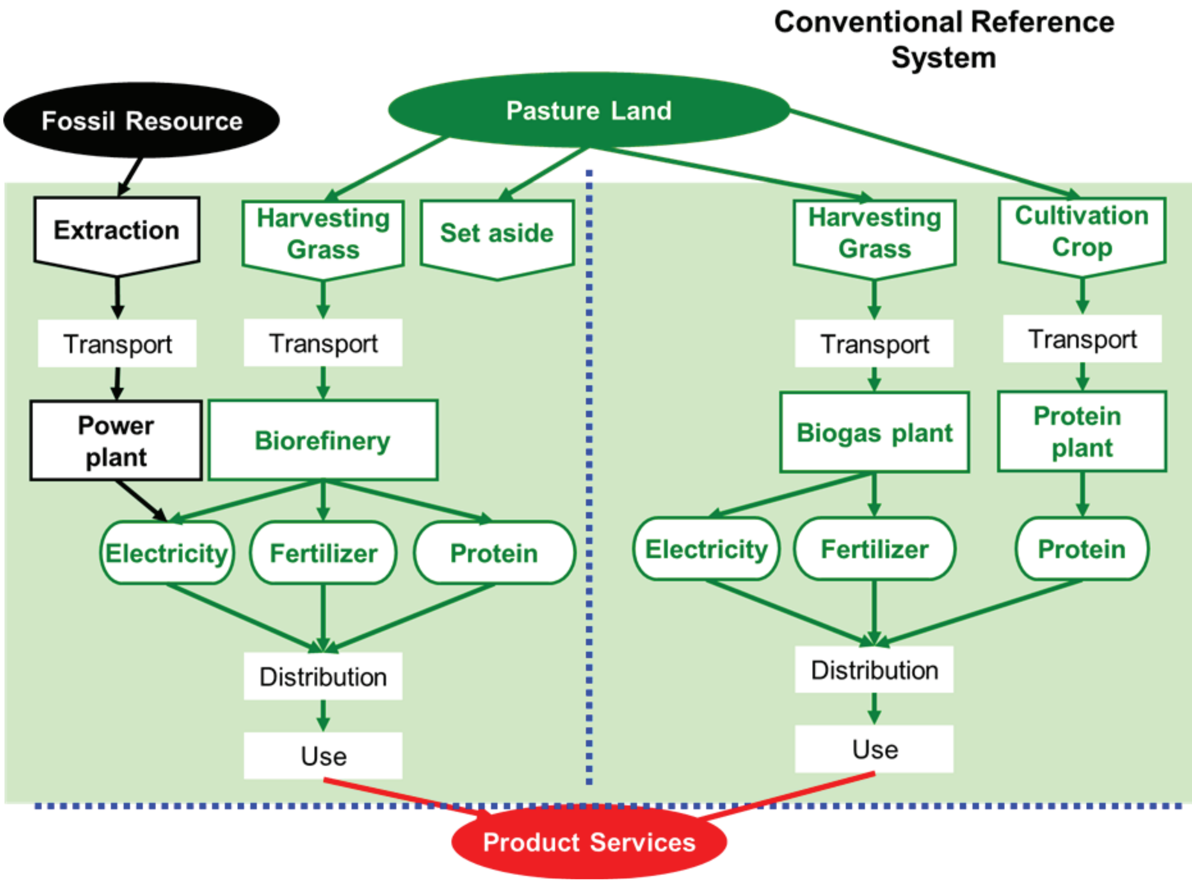


Figure 26. *Comparison biorefinery with conventional reference-system on full value chain (incl. "end of life treatment")*

Table 21. *Key characteristics biorefinery value chain*

Whole value chain			
Greenhouse gas emissions		**range**	
biorefinery	3	(3.1 to 3.8)	[kt CO_2-eq/a]
reference system	4	(4.1 to 5.1)	[kt CO_2-eq/a]
saving	-24%	(-22% to -28%)	[%]
Cumulated energy demand			
fossil			
biorefinery	0.1	(0.05 to 0.06)	[PJ/a]
reference system	0.1	(0.07 to 0.09)	[PJ/a]
saving	-31%	(-29% to -36%)	[%]
total			
biorefinery	0.5	(0.43 to 0.54)	[PJ/a]
reference system	0.5	(0.49 to 0.61)	[PJ/a]
saving	-12%	(-11% to -13%)	[%]
Agricultural area demand			
feedstock	8,900	(8200 to 10200)	[ha/a]
Costs			
annual costs	3	(3 to 3.7)	[Mio €/a]
specific costs	46	(43 to 53)	[€/t]
Revenues			
annual revenues	4	(3.4 to 4.3)	[Mio €/a]
specific revenues	53	(50 to 61)	[€/t]

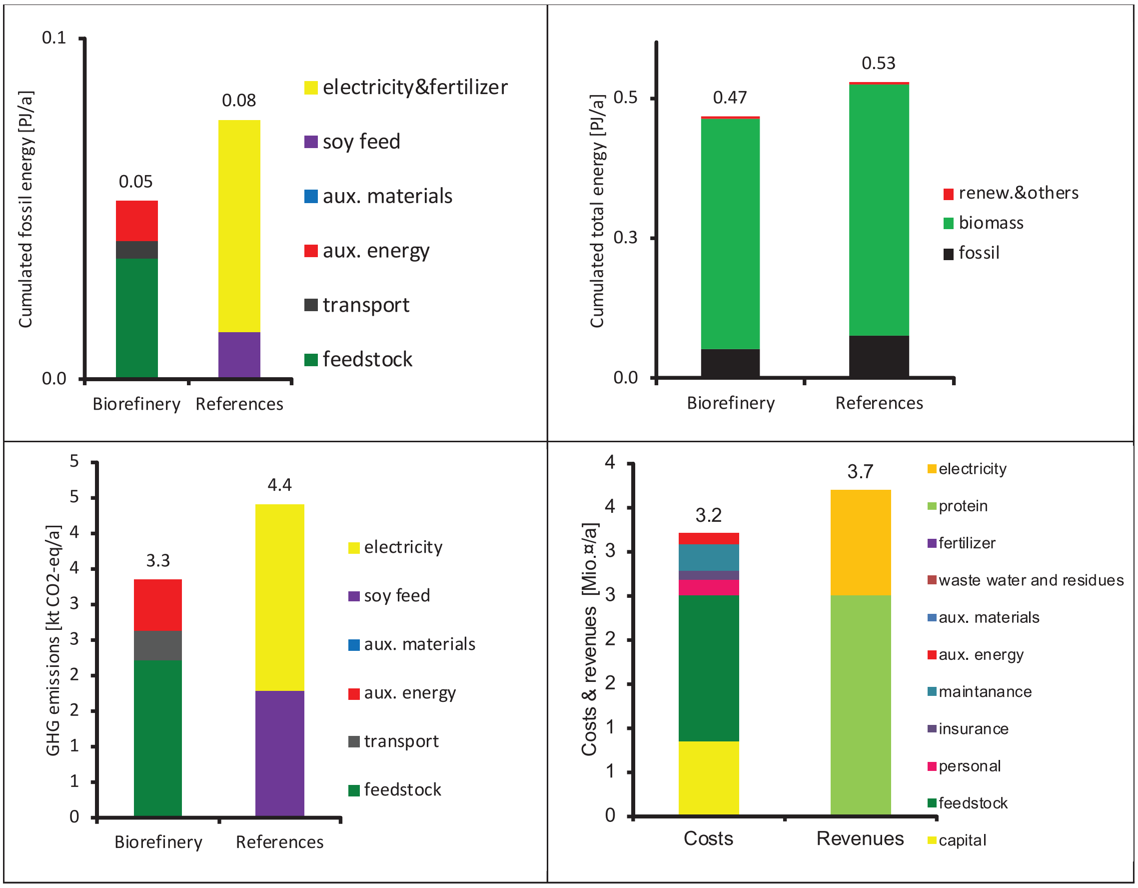
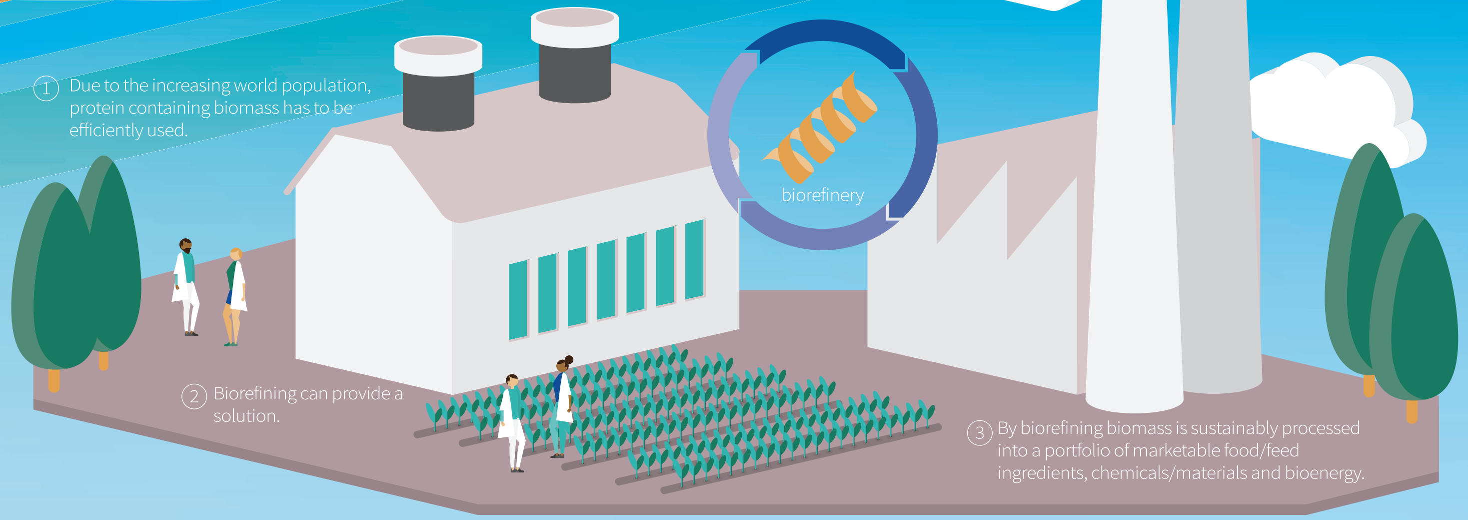


Figure 27. *Annual GHG emission, cumulated primary energy demand, cost and revenues of the full value chain*

Biorefining of protein containing biomass

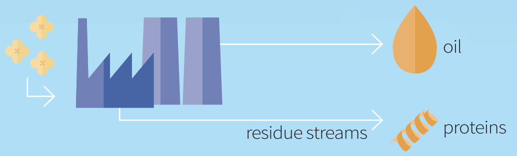


Oil crops

The four most important oil crops in Europe:

olives · rapeseed · sunflower seed · soybean

By refining of oil crops, oil production is combined with the production of protein-rich residue streams.

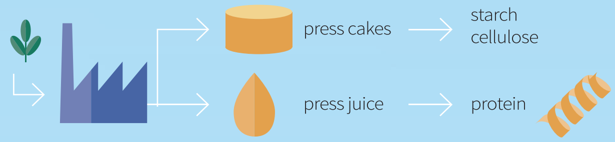


Herbaceous biomass

Herbaceous crops are primarily used as forage and are a source of leafy vegetables.

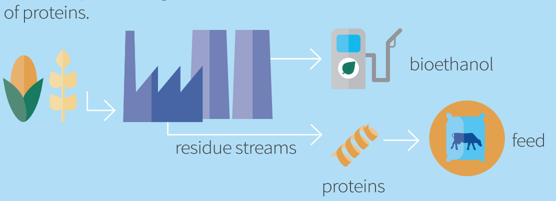
grass · beat leaves · lucerne

Refining generally will produce a fibre-rich press-cake and a protein-rich press-juice that both can be processed downstream to co-produce proteins, products and energy.



Dried distillers grains with solubles (DDGS)

Industrial processes generate residue streams that can contain certain amounts of proteins.

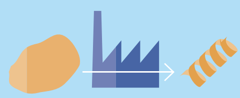


Starch crops

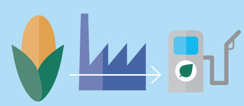
Starch crops are used as human food, but also as sources of starch, for the production of proteins and ethanol.

potato · grains and cereals · legumes

Biorefinery increases the functionality of protein in potato.



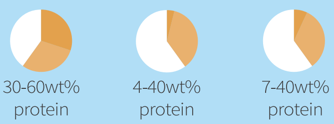
Biorefinery is used to produce bioethanol from maize.



Aquatic biomass

Aquatic biomass forms a new source of protein.

microalgae · macroalgae · duckweed



Biorefining of protein containing biomass co-producing protein-based biobased products and bioenergy offers the opportunity to result in market competitive business cases

References

www.indexmundi.com/commodities Ahamed, N.T., R.S. Singhal, P.R. Kulkarni and M. Pal. 1998. A lesser known grain, Chenopodium quinoa: review of the chemical composition of its edible parts. Food and Nutrition Bulletin 19, 61-70.

Alamanou, G. D. (1997). Effect of wet extraction methods on the emulsifying and foaming properties of lupine seed protein isolates (Lupinus albus ssp. Graecus). Food Hydrocolloids, 409–413.

Alting, L. P. (2011). Potato proteins. In Handbook of food proteins (pp. 316-334). Woodhead Publishing Limited.

Anyango, H. L. (2011). Evaluation of the functional quality of cowpea-fortified traditional African sorghum foods using instrumental and descriptive sensory analysis. LWT - Food Science and Technology, 2126–2133.

Arntfield, H. D. (2011). Peas and other legume proteins. In Handbook of food proteins (pp. 233-266). Woodhead Publishing Limited.

Arntfield, S. (2011). Canola and other oilseed proteins. In Handbook of food proteins (pp. 289-315). Woodhead Publishing Limited.

Becker, E.W. 2007. Micro-algae as a source of protein. Biotechnology Advances 25, 207-210.

Benjamin T. Wong, J. Z.-I. (2012). Conformational changes to deamidated wheat gliadins and β-casein upon adsorption to oil–water emulsion interfaces. Food Hydrocolloids, 91–101.

Bikker, P., M.M. van Krimpen, W. Brandenburg, A. López-Contreras, P. van Wikselaar and R.A. Dekker. 2014. Estimation of the nutritive value of seaweed species & extracted seaweed in animal diets. Presentation EAAP, session 52.

Christaki, E., M. Karatzia and P. Florou-Paneri. 2010. The use of algae in animal nutrition. Journal of the Hellenic Veterinary Medical Society 61, 267-276.

Chronakis, M. M. (2011). Algal proteins. In Handbook of food proteins (pp. 353-394). Woodhead Publishing Limited.

Cormont, A. en M.M. van Krimpen. 2014. Het percentage regionaal eiwit in het Nederlands veevoerrantsoen. Rapport Wageningen UR Alterra en Wageningen UR Livestock Research, Wageningen.

CVB. 2012. Tabellenboek Veevoeding 2012. Voedernormen landbouwhuisdieren en voederwaarde veevoeders. Productschap Diervoeder, Den Haag.

Day, L. (2013). Proteins from land plants – Potential resources for human nutrition and food security. Trends in Food Science & Technology.

Duodu, J. T. (2003). Factors affecting sorghum protein digestibility. Journal of Cereal Science, 117–131.

Dickinson, E. (2012). Use of nanoparticles and microparticles in the formation and stabilization of food emulsions. Trends in Food Science & Technology, 4–12.

Duranti, A. C. (2008). The major proteins of lupine seed: Characterisation and molecular properties for use as functional and nutraceutical ingredients. Trends in Food Science & Technology, 624–633.

Egaña, J. I., Uauy, R., Cassorla, X., Barrera, G., & Yañez, E. (1992). Sweet lupine protein quality in young men. The Journal of nutrition, 2341-7.

Fefac. 2013. Feed & Food Statistical Yearbook 2005-2012

Fredrikson, M. M. Larsson Alminger, N.G. Carlsson & A.S. Sandberg. 2001. Phytate content and phytate degradation by endogenous phytase in pea (Pisum sativum). J. Sci. Food Agric. 81, 1139-1144.

Fukushima, D. (2011). Soy proteins. In Handbook of food proteins (pp. 210 232). Woodhead Publishing Limited.

Gonzalez-Perez, S., K. B. Merck, J. M. Vereijken, G. A. van Koningsveld, H. Gruppen, and A. G. J. Voragen. 2002. Isolation and characterization of undenatured chlorogenic acid free sunflower (Helianthus annuus) proteins. Journal of Agricultural and Food Chemistry 50, 1713-1719.

Handbook of food proteins. (2011). Woodhead Publishing Limited.

Helsper, J.P.F.G., A. Balkema-Boomstra, S.A. Ribôt, M.J. Groot and

Hirose 2003. Egg white proteins – ovalbumin. In Industrial Protein in Perspective (W.Y. Aalbersberg et al., eds) Elsevier, Amsterdam, The Netherlands, pp. 40-43.

Innova - Food and Beverage Innovation. (2015, June). *Volume 13 (5-2015)*.

Innova Database. (2015). Retrieved from http://www.innovadatabase.com.

Kamp, J., van Berkum, S., Timmer, R. and van Reeuwijk, P. 2010. Verkenning naar de mogelijkheden van eiwithoudende teelten in Europa Rapport in opdracht van ministerie LNV. PPO publicatienummer 412.

Krimpen, M.M., P. Bikker, I.M. van der Meer, C.M.C. van der Peet-Schwering and J.M. Vereijken. 2013. Cultivation, processing and nutritional aspects for pigs and poultry of European protein sources as alternative for imported soybean products. Report 662, Wageningen UR Livestock Research, Wageningen.

Lamont, J. and Y. Lambrechts. 2005. Koolzaad, van zaad tot olie. Brochure van het Ministerie van de Vlaamse Gemeenschap.

Liener, I.E. (1989) Antinutritional factors in legume seeds: State of the art. In: J. Huisman, A.F.B. van der Poel & I.E. Liener, edsRecent Advances of Research in Antinutritional Factors in Legume Seeds and Rapeseed. Proceedings of the first International symposium on Antinutritional factors (ANF) in legume seeds. PUDOC, Wageningen, The Netherlands, pp. 6-13.

Loo, van. 2006. Novel protein crops as pig feed in organic farming. Report 112, Plant Research International, Wageningen.

Mawson, R., R.K. Heaney, Z. Zdunczyk and H. Kozlowska. 1994. Rapeseed meal glucosinolates and their antinutritional effect. Part 5. Animal reproduction. Nahrung, 38, 588-598.

Mulder, W. 2010. Proteins in Biomass Streams. Report 1134 'Platform Groene Grondstoffen'.

Pol van den-Dasselaar, A., D. Durksz, A. Klop J.M.J. and Gosselink. 2012. Grasraffinage in de Veehouderij. Rapport 556, Wageningen UR Livestock Research, Wageningen.

Pribis, B. S.-H. (2014). Cognition: the new frontier for nuts and berries. American journal of clinical nutrition, 347S-352S.

Rollema, 2003. Caseiantes. In Industrial Protein in Perspective (W.Y. Aalbersberg et al., eds) Elsevier, Amsterdam, The Netherlands,, pp. 255-259

Salgado, P. R., S. E. M. Ortiz, S. Petruccelli, and A. N. Mauri. 2011. Sunflower Protein Concentrates and Isolates Prepared from Oil Cakes Have High Water Solubility and Antioxidant Capacity. Journal of the American Oil Chemists Society 88, 351-360.

Schöne, F. G. Jahreis, R. Lange, W. Seffner, B. Groppel, A. Henning and H. Lüdke. 1990. Effect of varying glucosinolate and iodine intake via rapeseed meal diets on serum thyroid hormone level and total iodine in the thyroid in growing pigs. Endocrinology Exp., 24, 415-427.

Spruijt, J., R. van der Weide en M. van Krimpen. 2014. Kansen voor micro-algen als grondstofstroom in diervoeders. Rapport PPO nr. 619. ACRRES-Wageningen UR, Wageningen.

Stegeman, D., A.M. Janssen, J.P.F.G. Helsper, I.M. van der Meer and H.R.J. van Kernebeek. 2010. Technologie en grondstoffen voor vleesvervangers en hoogwaardige eiwitten. Rapport BO-08-008-002 in opdracht van ministerie van LNV.

Tusche, S. A. (2012). Wheat gluten and potato protein concentrate — Promising protein sources for organic farming of rainbow trout (Oncorhynchus mykiss). Aquaculture, 120–125.

Vahl, H. 2009. Alternatieven voor Zuid-Amerikaanse soja in veevoer. Rapport Vahl Feed and Health.

Veldkamp, T., G. van Duinkerken, A. van Huis, C.M.M. Lakemond, E. Ottevanger, G. Bosch and M.A.J.S. van Boekel. 2012. Insects as a sustainable feed ingredient in pig and poultry diets - a feasibility study. Report 638, Wageningen UR Livestock Research, Wageningen.

Waglay, S. K. (2014). Potato protein isolates: Recovery and characterization of their properties. Food Chemistry, 373-382.

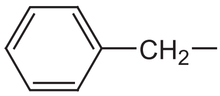
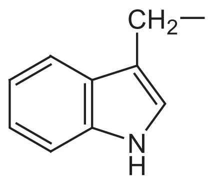
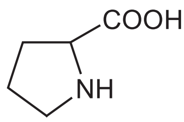
WHO/FAO. (1991). Protein quality evaluation. Report of the Joint FAO/WHO. Expert Consultation. Auckland.

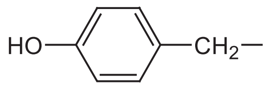
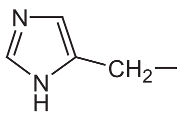
WHO/FAO/UNU. (2007). Protein and amino acid requirements in human nutrition, Report of a joint FAO/WHO/UNU expert consultation (WHO Technical Report Series 935).

Wijffels, R.H. and M.J. Barbosa. 2010. An outlook on microalgal biofuels. Science 329, 796-799.

www.dmv.nl; www.Fonterra.com; www.Henningsenfoods.com; www.Bouwhuis-Enthoven.com; www.NIVE.nl; www.voederwaardeprijzen.nl

Annex 1. The twenty alpha amino acids that occur in proteins

Name	Abbreviation		R	Isoelectric point (pI)
Apolar side chain				
Glycine	Gly	G	H—	5.97
Alanine	Ala	A	CH_3-	6.00
Valine	Val	V	CH_3 $\rangle CH-$ CH_3	5.96
Leucine	Leu	L	CH_3 $\rangle CH-CH_2-$ CH_3	5.98
Isoleucine	Ile	I	CH_3CH_2 $\rangle CH-$ CH_3	6.02
Phenylalanine	Phe	F		5.48
Tryptophan	Trp	W		5.89
Proline	Pro	P	 (whole structure)	6.30

Side chains with a hydroxyl group				
Serine	Ser	S	$HO-CH_2-$	5.68
Threonine	Thr	T	$HO-CH(CH_3)-$	5.64
Tyrosine	Tyr	Y		5.66
Side chains with a carboxyl group				
Aspartic acid	Asp	D	$HOOC-CH_2-$	2.77
Glutamic acid	Glu	E	$HOOC-(CH_2)_2-$	3.22
Side chains with an amide group				
Asparagine	Asn	N	$H_2N-C(=O)-CH_2-$	5.41
Glutamine	Gln	Q	$H_2N-C(=O)-(CH_2)_2-$	5.65
Side chains with a basic group				
Lysine	Lys	K	$H_2N-(CH_2)_4-$	9.74
Histidine	His	H		7.59
Arginine	Arg	R	$H_2N(HN=)C-NH-(CH_2)_3-$	10.76
Side chains with a sulfur containing group				
Cysteine	Cys	C	$HS-CH_2-$	5.07
Methionine	Met	M	$CH_3-S-(CH_2)_2-$	5.74

Annex 2. The structure of proteins

Primary structure:

sequence of amino acids in the chain

Secondary structure:

the fold of the polypeptide chain which is maintained by the hydrogen bonds between the amide groups

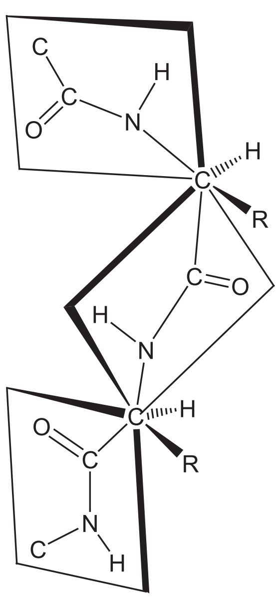
Tertiary structure:

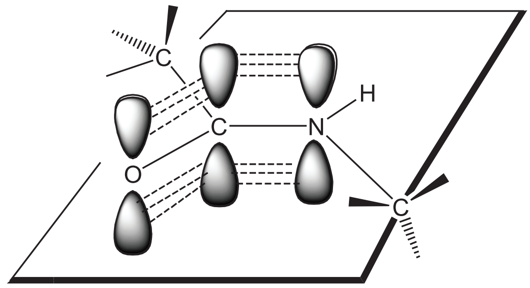
the fold of the individual secondary structures in 3D, mainly maintained by interactions between the amino acid side chains

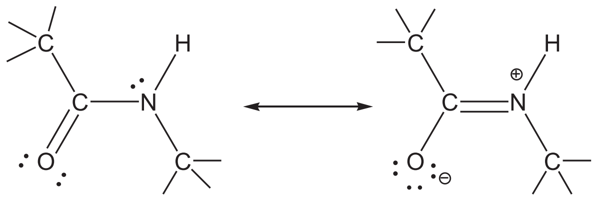
Quaternary structure:

the 3D-structure of several polypeptide chains (and nonproteinaceous parts)

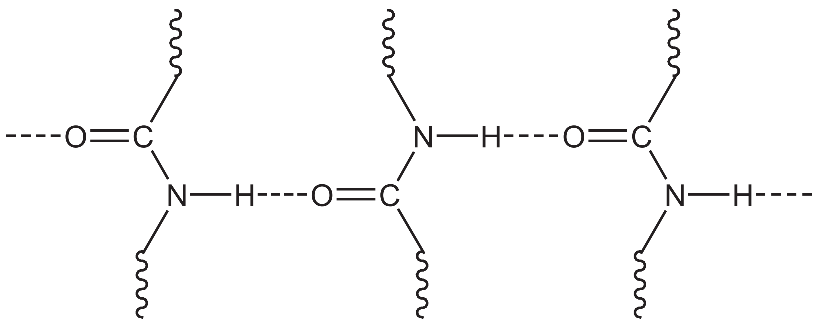
Secondary structure



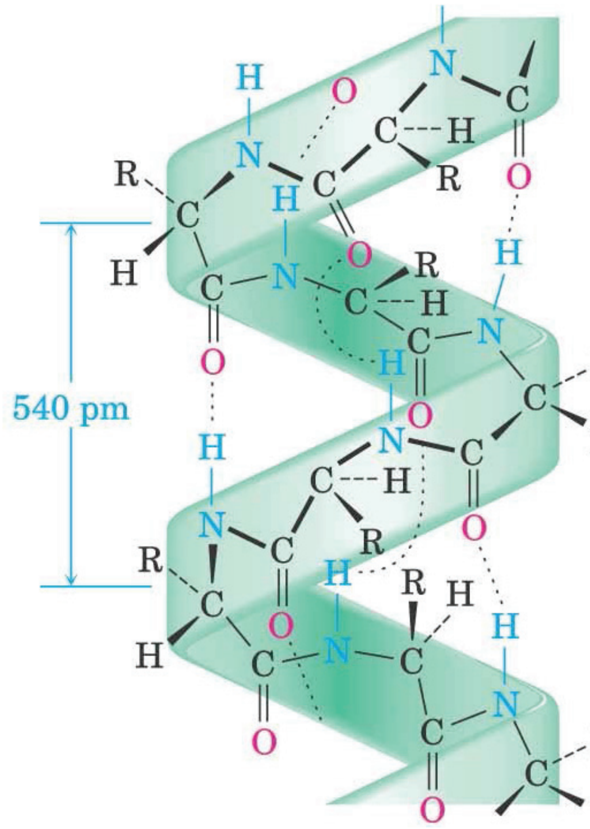


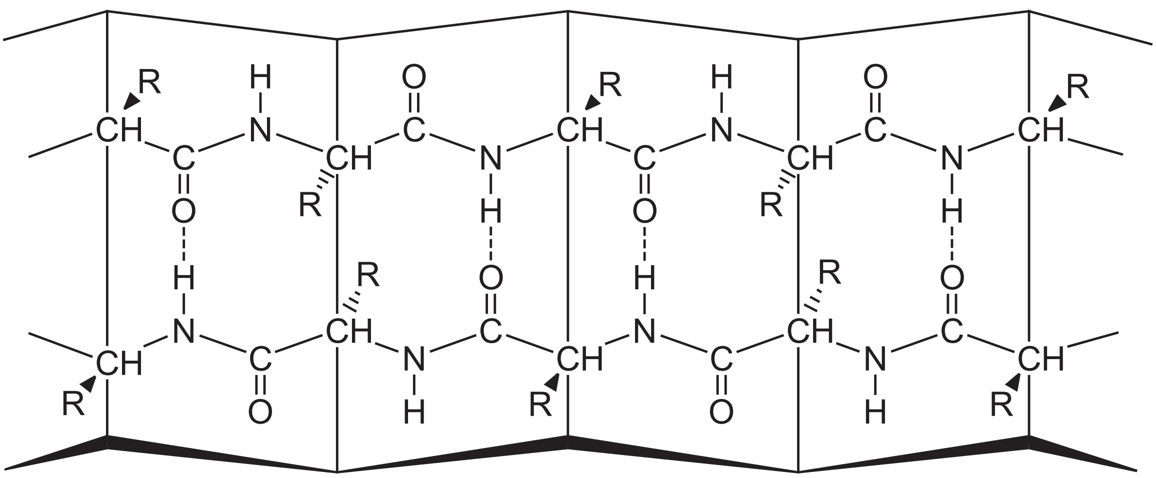


Trans orientation of the chain around the peptide bond



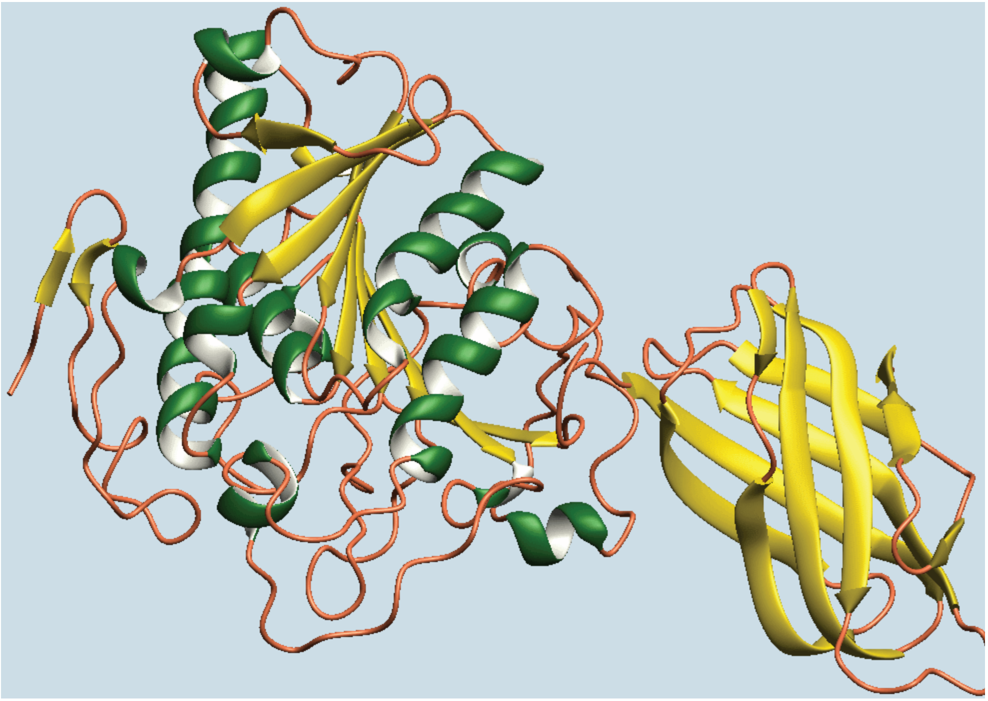
Example of a secondary structure





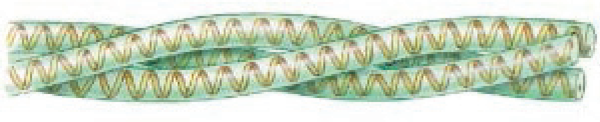
the antiparallel β-sheet structure

Example of tertiary structure



Orange: random coil
Yellow: β sheet
Green: α helix

Example of quaternary structure



(b) Keratin fiber

Acknowledgements

Maria Wellisch – Agriculture and Agri-Food Canada / Government of Canada, Verna Mitura - Grains & Oilseeds Division, Agriculture and Agri-Food Canada / Government of Canada, Janitha Wanasundara - Research Scientist, Science and Technology Branch Agriculture and Agri-Food Canada / Government of Canada, Geoff Bell – Microbiogen Pty Ltd/Australia.

Further, the authors specifically do want to thank Dr. Gerfried Jungmeier from Joanneum Research GmbH (AT) for his contribution in the preparation of the protein-related biorefinery fact sheets (BFSs).

IEA Bioenergy

Further Information

IEA Bioenergy Task42 Website
www.iea-bioenergy.task42-biorefineries.com

IEA Bioenergy Website
www.ieabioenergy.com