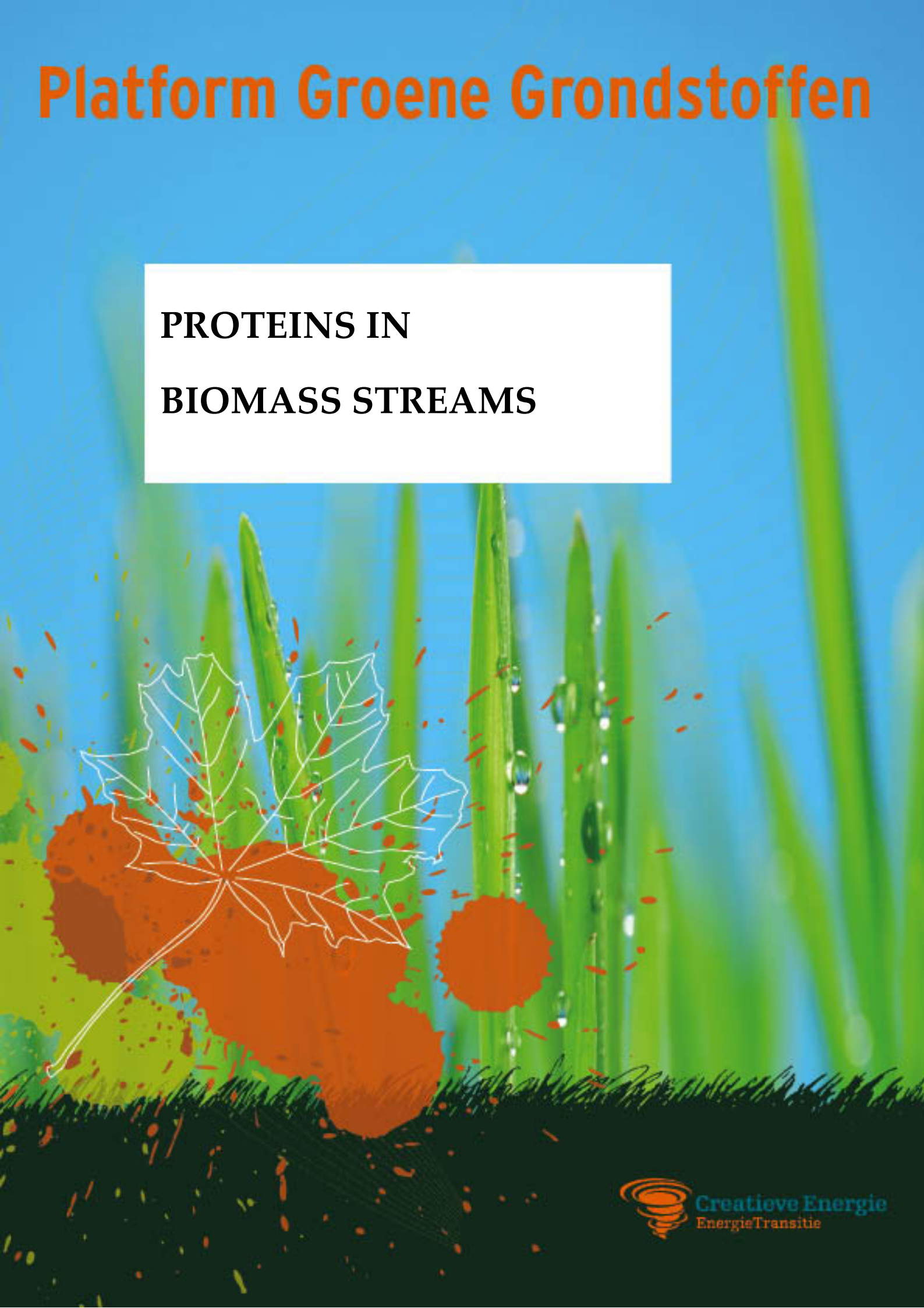
Platform Groene Grondstoffen

PROTEINS IN BIOMASS STREAMS



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PROTEINS IN

BIOMASS STREAMS

A study commissioned by the Biorenewable Resources Platform (Platform Groene Grondstoffen)

April 2010

Colophon

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1 Introduction

Proteins are found in nature in a variety of forms and are of crucial importance in the structure and function of living cells. Proteins play a variety of very specific roles in:

- regulating processes (hormones, gene expression, antibodies for the immune system);
- enzymatic catalysis (enzymes);
- stabilisation of cell walls, skin, and bone and;
- organising transport of materials and storage of energy.

Their principal structural elements are polypeptide chains, although they may be combined with fats as lipoproteins and with polysaccharides as glycoproteins. Proteins have complex structures based on their amino acid composition, three dimensional structures (helices, beta sheets) and the way subunits are linked together (see Appendix 1). Molecular weights vary from thousands to millions Dalton. The molecules may consist of one single chain or two or more chains joined by disulfide bonds. Globular proteins consist of chains tightly intertwined to form a nearly spherical shape. In some more complex proteins these spherical units may themselves be joined together by non-covalent forces into larger structures of fairly precise form.

Proteins can be found in several agro-materials, plants and animals. Proteins play an important role in the diets of animals and humans. Traditionally, for food consumption cereals (e.g. wheat, barley and sorghum) and legumes (green peas, lentils, beans and chick peas) are being grown. A number of proteins have been produced commercially for a long time. These proteins, such as soy proteins, casein, whey protein and wheat gluten, are being used both in food and non food area.

Recent years, new biomasses, such as algae and grass, containing proteins have become subject of studies. Apart from these new proteins resources, also biorefinery research programmes are being carried out focussing on the isolation of valuable compounds, like proteins, from varies industrial waste streams. As an example, the recovery oil from oilseeds like sunflower seeds or rapeseeds leaves behind press cakes with a high protein content.

Scope of this study:
The focus of this study is to give an overview of traditional and new biomasses and biomass streams that contain proteins. When information was available, the differences in molecular structure and physical and chemical properties for the different proteins is given. For optimal biomass use, isolation of valuable compounds like proteins can be an important aspect. To make decisions possible if biorefinery strategies by which the isolation of proteins is feasible, the economical value and production volumes of the different biomass streams will be discussed (when available). Also the industrial relevance and possible applications, such as technical applications and chemicals derived from proteins, will be reported. In addition, the outlet of protein-rich biomass resources in the feed sector will be pointed out. Protein (sources) that are probably *only* relevant as food (ingredient), such as mushrooms, insects and legumes are not part of this study.

2 Traditional proteins resources and isolation processes

Agricultural products can contain substantial amounts of proteins. A number of proteins, both derived from plants and animals, have been produced commercially for a long time. These proteins, such as potato proteins, proteins from cereals, soybeans and milk, collagen en gelatine, are being used in food and feed, as well as in various non-food or technological applications.

In the following paragraphs, the most important aspects of the traditional proteins are being described. First, wheat gluten will be discussed since nomenclature of the different protein fractions in relation to their solubility is identical to some other proteins. Next, soy proteins structure and its extraction process from soy beans is being discussed in detail because (i) soy proteins have been produced commercially on large scale for long time and many aspects have been studied, and (ii) the isolation process can be considered as a standard process, also partly applicable for other extraction of proteins from other biomasses.

2.1 Wheat proteins

Wheat consists of a range of proteins. Osborne divided wheat proteins into four categories, according to their sequential extraction from wheat flour. The first water-soluble protein fraction from defatted and crushed seeds consists of the albumins (3-5%). Next are the globulins (6-10%), obtained by washing in dilute salt. That fraction is followed by ethanol fractionation, called the gliadins (40-50%). The insoluble remainder consists of glutenins (30-40%). Gluten is the main storage protein in wheat. Wheat is an important cereal crop being used in bakery products and other food applications. Gluten may be defined as the insoluble mass which remains when wheat flour dough is washed to remove starch and water-soluble components (Figure 1). In fact, gluten is a side product from the starch industry. The poor solubility of wheat gluten in water and its cohesive properties are used to separate it from wheat starch (Figure 2). First a slack 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, hydrocyclones 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 glutenparticles 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 hydrocyclones, concentrated in decanters and subsequently dried or modified. Most of the process water is recycled. Some of it is concentrated together with by-products, such as fibers and gums, and used as animal feed.

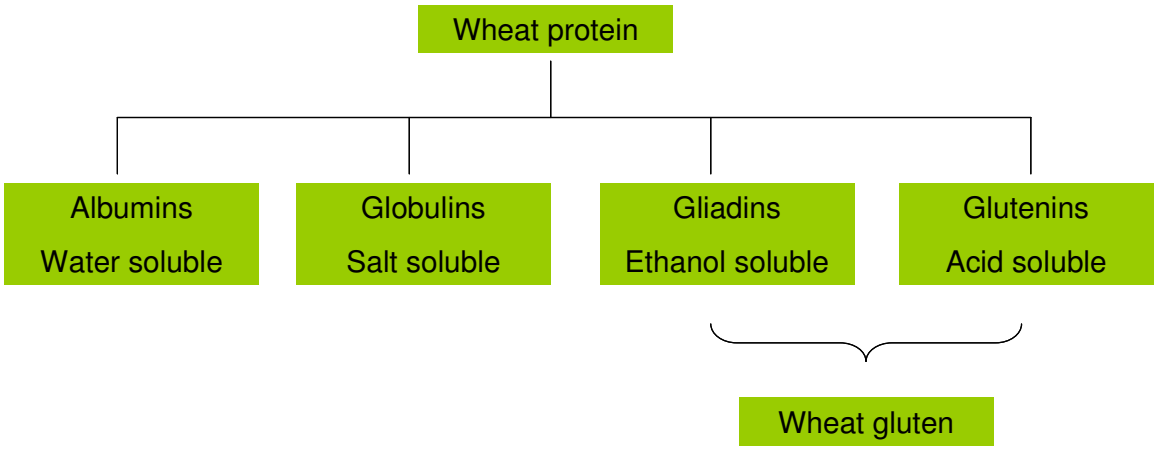


Figure 1. *Different protein fractions in wheat protein based on their solubility in different solvents*

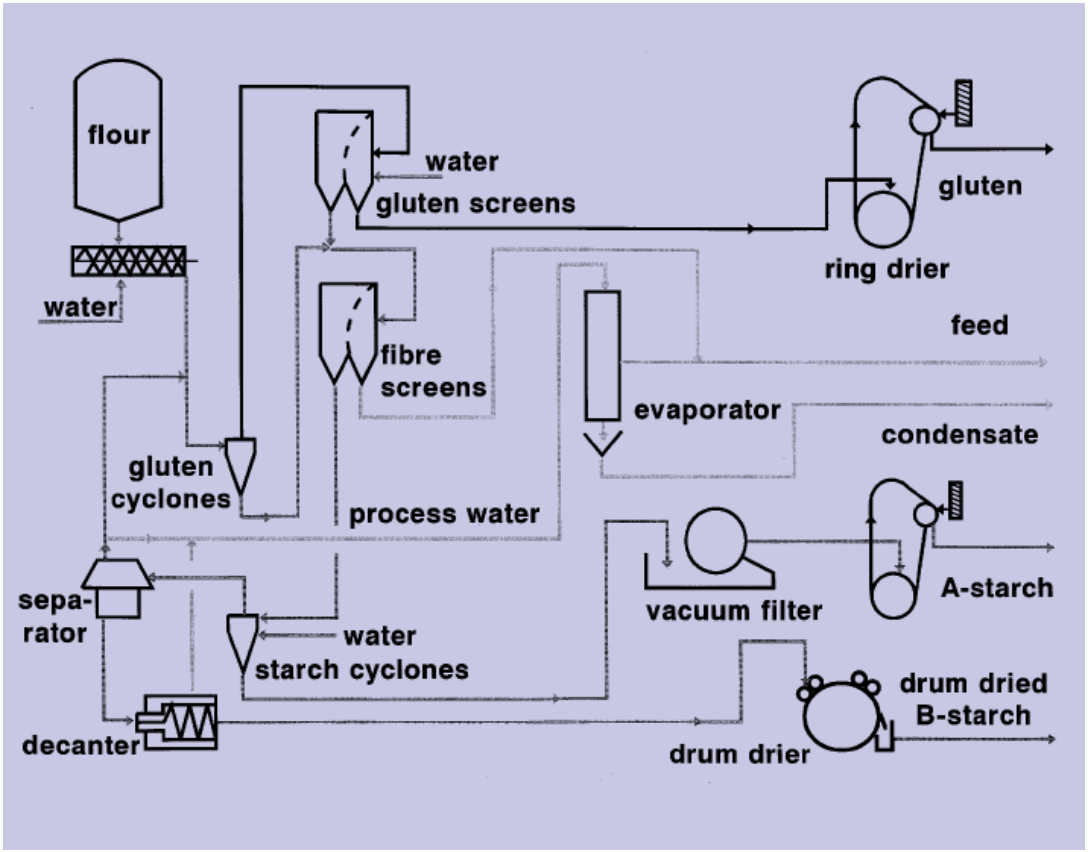


Figure 2. *The starch/gluten separation process*

Gluten is a cohesive material with two important physical properties; elasticity and extensibility. This combination of properties, which is unique among proteins, is largely responsible for the fact that wheat dough can be baked into various bakery products. Wheat gluten is composed mainly of 55 wt.% of glutenins (polymeric and elastic) and 45 wt.% of gliadins (monomeric and viscous). Both fractions consist of water-insoluble proteins. Glutenins consist of many subunits linked together by intermolecular disulphide bonds. These subunits can be divided into two main groups: high molecular weight and low molecular weight subunits. The gluten complex is believed to be a protein network held together by extensive covalent and non-covalent bonding (Figure 3). The elastic and cohesive character of gluten is due to a great extent to the presence of disulfide bonds.

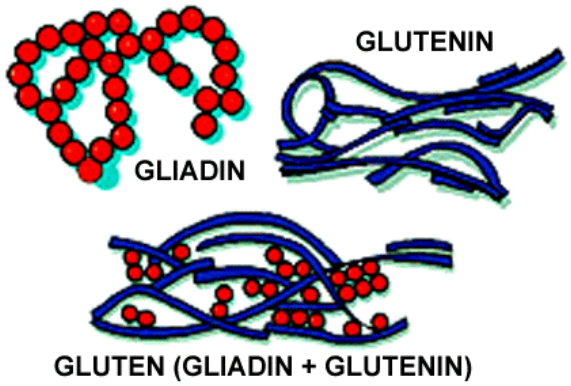


Figure 3. *Network formation of wheat gluten*

Films based on wheat gluten are not water soluble and have been used for encapsulation in order to improve the quality of cereal products and retain microbial or antioxidant additives on food surfaces. Compared with other proteins, gluten has high gas-barrier properties (mainly O_2 and CO_2). Wheat gluten has mostly found applications in adhesives, coatings, and cosmetics.

2.2 Soy protein

Soybeans have a high protein content of about 40% (30% carbohydrates, 20% oil, 5% fibre and 5% ash on dry basis) and are transformed into different soy proteins products for use in food, feed and also non-food applications. Soy proteins are commercially available as soy flour, soy concentrate and soy isolate, all differing in protein content. The soybean contains a variety of proteins. Based on their solubility, soy proteins are divided into two classes: albumines (water soluble) and globulines (soluble in salt solutions). The majority of the soy proteins (about 90%) are globulines, which further divided in soybeans as glycinins and conglycinins,.

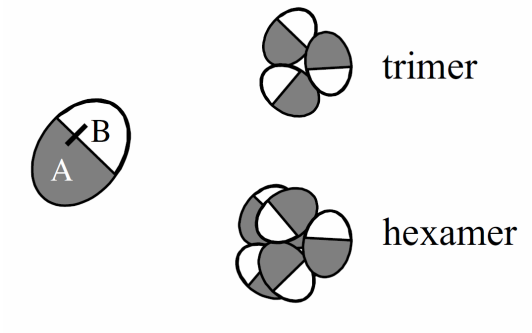


Figure 4. *Schematic presentation of a glycinin molecule and its trimeric and hexameric complexes. A and B represent the acidic and basic polypeptides, respectively. The connection bar between A and B represents a disulphide bond.*

Table 1. *protein composition of extractable soy protein*

Sedimentation coefficient	**components**
2S	trypsin inhibitors, cytochrome C, α-conglycinin
7S	β- and γ-conglycinin, lipoxygenases, α-amylases, hemagglutenins
11S	glycinin
15S	polymers of glycinin

Besides the classification related to solubility, another classification is based on sedimentation coefficients using ultracentrifugation to separate proteins (Table 1). Using this technique four fractions increasing in molecular weight can be distinguished: 2S (20%), 7S (37%), 11S (33%), and 15S (10%). Only the 11S and the 15S fractions are pure proteins; they are comprised of glycinin and polymers of glycinin, respectively. The 11S glycinin is the hexamer having a molecular weight of about 360 kDa. Each of the six subunits is composed of an acidic polypeptide of about 35-40 kDa and a basic polypeptide of about 20 kDa. The basic and acidic polypeptide are linked by a disulfide bond. Glycinin is considered to be a dimer of two trimers. The dimer dissociates to trimers depending on conditions of pH, ionic strength and temperature.

The majority in 7S is β- and γ-conglycinin and in 2S α- conglycinin. β-conglycinin is the most prevalent, it is a trimer with a molecular mass of 150-200 kDa. The three subunits have masses of respectively 72 kDa, 86 kDa and 53 kDa. The three dimensional structure of β-

conglycinin is unknown. However, these structures are known for the strongly related vicilins phaseolin (Figure 5).

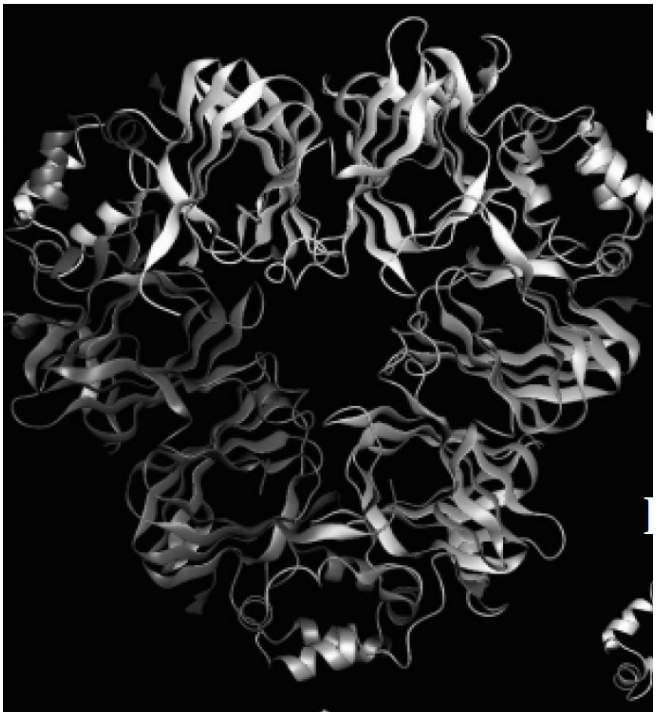


Figure 5. *Structure of the French bean protein phaseoline, which has a comparable structure as soybean β-conglycinin*

Processing soy proteins affects their solubility and biological activity. The native proteins in principle are all water soluble. In the process of extracting the oil and subsequent heat processing, some of the proteins lose their solubility in water. The extent of denaturation, inactivation and solubility loss is related to the intensity of heat treatment given to the product. Processing of soybeans involves drying, cracking, dehulling and rolling into flakes. These flakes are either milled to yield full fat soy flour or extracted with hexane to remove the oil. Residual hexane is stripped away with steam and removed either via a desolventiser toaster or in a flash desolvantiser system. Extracted soybean flakes are processed into standard meal for animal feed or into special protein products. Steam is used to remove residual hexane from the flakes. The use of a desolventiser toaster tends to darken the meal due to Maillard reactions. Protein levels are generally between 45-50%. To obtain material with a higher protein content than 50%, it is necessary to remove some of the soy components other than oil, like sugars and minerals. Soy concentrates and isolates are obtained after additional processing to bring protein levels to about 70% or more than 90% on dry base. The majority of the concentrates are produced through aqueous alcohol extraction. Isolates are obtained by dissolving the protein, followed by iso-electric precipitation.

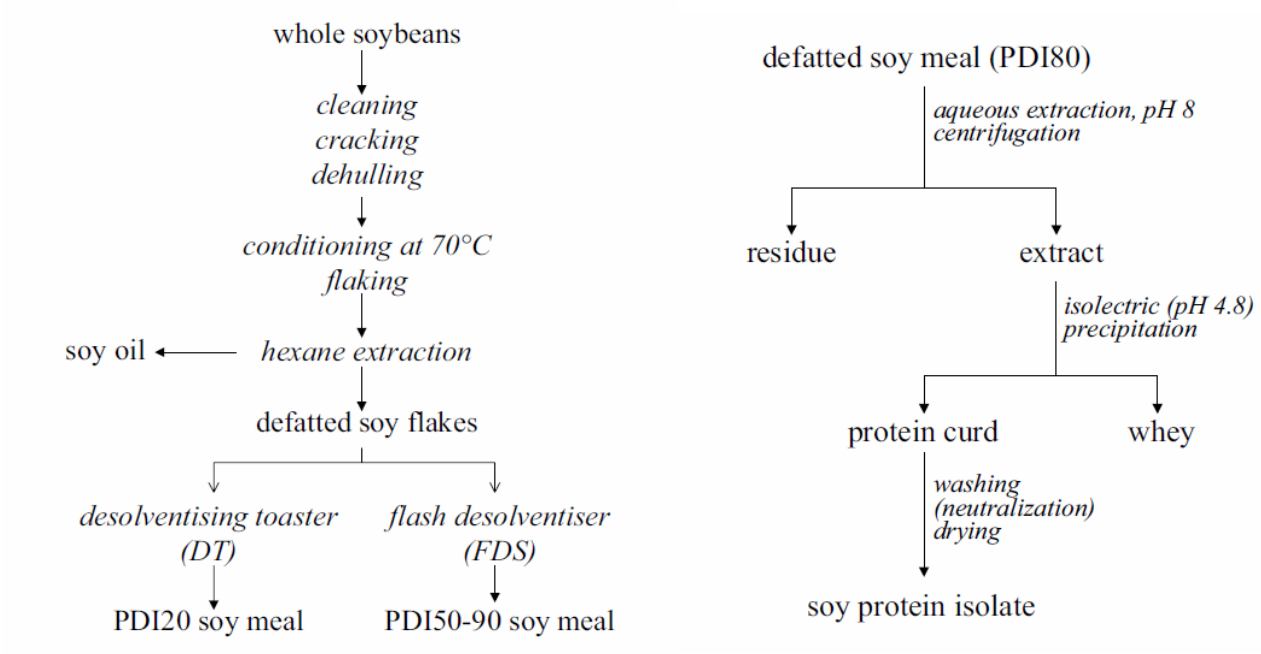


Figure 6. *Production of defatted soy meal (left) and soy protein isolate (right). PDI: Protein Dispersibility Index*

There has been an increasing interest in the use of soy protein for a range of applications, including paper coatings; plywood adhesives; and microencapsulation of insecticides, food ingredients, and pharmaceutics.

2.3 Milk proteins; casein and whey proteins

Much of the milk is directly consumed as milk and as dairy products. On average, milk has a protein content of about 3.5% protein , 1% minerals, 4.5% fat, 5% lactose and 86% water. The two major protein fractions are caseins (about 80%) and whey proteins (about 20%). The complete protein composition of milk is depicted in Table 2.

Table 2. *Concentration of proteins in milk (g/L)*

Caseins		**Whey proteins**		**Miscellaneous**	
αs1 casein	10.0	β lactoglobulin	3.2	lactoferrin	0.1
αs2 casein	2.6	α lactoglobulin	1.2	transferrin	0.1
β casein	9.3	serum albumin	0.4	membrane	0.6
κ casein	3.3	proteose peptone	0.8		
γ casein	0.8	immoglobulin G1 and G2	0.7		
		immunoglobulin A	0.1		
		immunog;obulin M	0.1		

Caseins are predominantly phosphoproteins that precipitate at pH 4.6 (20°C) and are characterized by an open, random coil structure. The four major components that have been described are α_{s1}-, α_{s2}-, β- and κ-casein. These proteins contain several phosphorylated serine residues, some of which are clustered. Caseins are versatile and highly essential for the structure, texture, taste of diary products.

Caseins and the salts of caseins play also an important role outside the dairy industry. Casein is easy processable due to its random coil structure. By treating acid-precipitated caseins with alkali solutions, caseinates are produced. Caseins and caseinates form transparent and flexible films from aqueous solutions without treatment because of their random coil nature and numerous hydrogen bonds. It is not surprising that caseins have been shown to be useful proteins for encapsulation, namely for food and aquacultural applications. Currently, casein based labelling adhesives are being used in the bottle-labelling industry because of their very good runability at high machine speeds.

Whey proteins are by-products from the cheese production and are particularly rich in β-lactoglobuline. Whey protein concentrates (WPC) and isolates (WPI) are mainly used because of their nutritional value. Whey protein concentrates have a protein content of about 75-80%, whereas whey isolates have a protein content between 90 and 95%. WPCs are produced by partial removal of milk sugar (lactose) and milk minerals from whey. The partial delactosed whey is further purified dialysis and filtration processes.

Whey proteins are available in large amounts world wide and have been investigated as edible coatings and films. Whey proteins are characterized by water solubility at pH 4.6. Because of their globular nature, the production of films requires heat denaturation to open the globular structure, break existing globular disulfide bonds, and form new disulfide and hydrophobic bonds. Whey is a protein often used as an encapsulating material. However, due to the high price, other technical applications are not known.

2.4 Collagen and gelatine

Collagen is a fibrous, structural protein that is found in animal tissue, particular skin, bones and tendons. Because collagen may represent more than 90% of the organic mass of these tissues, the material can easily be isolated and collagen based tissues are readily available. Therefore, collageneous materials, as biomedical engineering materials are quit well investigated. Collagen is a flexible polymer, a long stretched protein with a molecular weight of about 285 kDa. The basic structure is a triple helix made up from three polypeptide chains (Figure 7). Due to its complex helical and fibrous structure collagen is very insoluble and difficult to process. Collagen has a common repeating unit: glycine, proline and hydroxyproline.

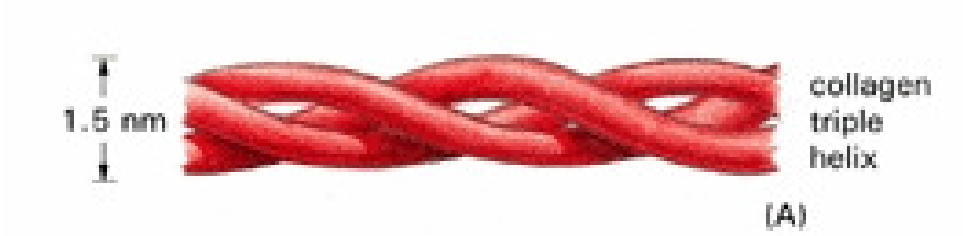


Figure 7. *Structure of collagen*

Collagen is the raw material for the production of gelatin, a common food additive. Gelatin is produced by a combined procedure of chemical and thermal treatments of collagen (Figure 8). The major sources for gelatin are pig skin and bones from cowes. Two main types of gelatin can be distinguished; type A which is produced by acid hydrolysis of collagen and type B, an alkaline processed collagen. Such treatments disrupt the tight helical structure of collagen and produce water-soluble fragments that may form stiff gels, films, or light foams.

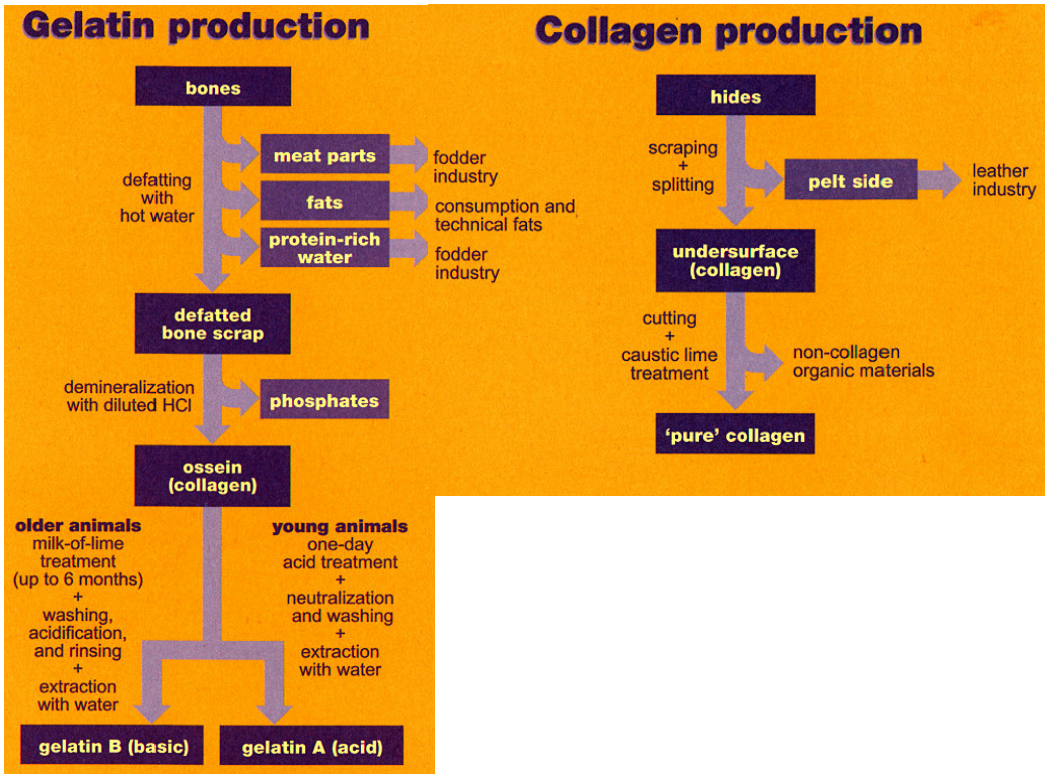


Figure 8. *Production of collagen en gelatin*

Gelatin is a well- processable material. Due to the isolation process, gelatin has a broad molecular weight distribution between 15 and 250 kDa with an average of 50-70 kDa. Gelatin dissolves in hot water, however, on cooling a gel is formed. This unique characteristic has triggered the development of gelatin capsules which are frequently used for the oral administration of drugs. Gelatin capsules rapidly swell in the gastro intestinal tract thereby releasing the active drug. Therefore, gelatin is extensively applied in the biomedical and pharmaceutical fields mainly due to their high biocompatibility.

2.5 Maize protein

Maize (Zea mays) is a major source for industrial starch production, and proteins are recovered as byproducts. Most of the corn starch is produced by the wet milling process. The primary objective of the wet milling is the production of high quality starch for food and industrial (e.g. pharmaceutical, chemical, paper industry). By wet milling, almost all the other components of maize, i.e. oil, fibres and proteins are recovered in commercial valuable byproducts. The total amount of protein in corn is about 10%, from which about 80% is located in the endosperm (Figure 9). The rest, 20% of the protein, is divided over the germ and the bran. Maize proteins are classified by Osborne based on their solubility as albumin (extractable in water), globulin (extractable in salt solutions), zein (extractable in aqueous alcohol), glutelin (extractable in dilute alkali), and the residue. The maize proteins are

recovered in protein-rich fractions such as germ, cake, gluten feed and gluten meal and mainly used as feed. The most abundant proteins in the maize kernel, the zeins, are located in protein bodies. The variety of methods for extracting zein from maize endosperm has led to a complex nomenclature for these proteins. Based on SDS-PAGE separation and nucleotide sequencing of the genes, the zeins consist of different distinct fractions with molecular weight of respectively 10, 15, 16, 19, 22 and 27 kDa. Because of a low content of polar amino acids and a high content of nonpolar amino acids, corn zein is not water-soluble. Corn zein can be dissolved in aqueous alcohol solutions. Due to the absence of essential amino acids, zein has no value as animal feed. However, zein forms transparent films, is relative hydrophobic and has potential in many technical applications. It has been used for example as coating material for pharmaceutical tablets.

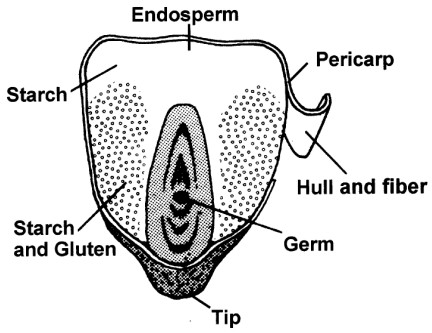


Figure 9. *Maize kernel*

2.6 Keratin

Keratin can be found in the tissues of vertebrates, such as skin, hair, feathers, nails, claws, hoofs, and scales. Since many of these materials can be found in waste streams, keratin is a cheap protein (Schrooyen 1999). For example, feathers are an important waste product of the poultry industry, causing an environmentally difficult disposal problem. Whole feathers may be used for clothing, and as insulation material. Feathers, consistingt mainly of keratin, is a potential feed source, however is very hard to digest. Therefore, the proteins need to be hydrolysed first. After collection from a processing plant, feathers are washed with water and cooked under pressure with constant agitation, after which the product is dried and ground to a powder, so called feather meal. This is used as an additive to animal feed.

Keratin has a high amount of cystine which can form disulfide bond. Due to the formation of these bonds, the protein is highly cross-linked and as a result very insoluble in water and therefore difficult to process. Extensively cleavage of the disulfide bonds is necessary to extract and isolate keratin. Various approaches, such as reduction and oxidation, have been used to extract and dissolve keratin. Anker developed a method to isolate keratin by using alkaline solutions. The molecular weight varies between 10,000 and 70,000 kDa. This process, however, can not be considered as a commercial process. Keratin has a high content of proline, serine, and glycine and a low content of lysine, histidine, methionine, and tryptophan.

2.7 Pea protein

A fairly new protein is pea protein. Pea is long and established and significant crop in Europe, but its production has been rather low for a long time. The increasing need of proteins both for animal feed and human nutrition resulted in a bigger interest in this crop. Pea seeds contain about 25 wt.% of protein. In general, the properties and composition of pea protein are quite similar to those described for soy protein. Pea proteins are mainly composed of water soluble proteins, including the globulin (55-65%) and the albumin (18-22%) fractions. Pea globulins are composed of two main families, legumin and vacilin, which belong respectively to the 11S and 7S storage proteins. Legumin has a molecular weight of 360 kDa and vacilin of 150 kDa. Albumins are defined as water soluble proteins. Pea lectines (50 kDa) are composed of two large (17 kDa) and two small subunits (5.7 kDa). Pea protein shows a well balanced profile of amino acids, and especially high content in lysine, one of the essential proteins. The success of peas in animal feed is due to the combination of a protein-rich composition together with a significant starch content.

Pea protein products are prepared for food uses either by a dry or a wet process. In the dry process, seeds are treated with air resulting in a starch and protein fraction. The protein fraction represents about 25 to 30% of the flour and has a protein content between 50-60%, depending on the flour composition and process. The wet process produces protein materials with a higher protein content. During this process, the flour is dispersed in water. By adjusting the pH to 7-9, the proteins go in solution. The protein extract is clarified by centrifugation and the proteins are recovered by ultra filtration or iso-electric precipitation. Depending on the process, the protein content of the isolates is in the range of 85-90%, with a protein recovery between 70-85%. Compared to soybean proteins or wheat gluten, the production is still rather limited.

2.8 Potato protein

Potato is a major crop that mainly is produced for food consumption. But apart from this, about 1/3 is used for the industrial starch production. Potatoes contain about 1.5% of protein on a fresh weight based, therefore there is an annual production 50 thousand tons of potato protein. This ranks potato protein second to soy bean in the amount of protein produced per hectare. A classification of potato proteins can be made as follows:

- Patatin, represents 40-60% of all buffer-extractable potato proteins and consists of a family of of 43 kDa. glycoproteins with their isoelectric point of approximately pH5. The primary sequence of patatin (362) amino acids shows neither extended hydrophilic nor hydrophobic amino acids sequences. The positive and negative charges of the side chains are randomly distributed over the sequence.
- Different types of protease inhibitors having molecular weights between 10 and 25 kDa.
- 20-30% are other proteins, like high molecular weight proteins involved in starch synthesis such as an 80 kDa phosphosporylase

After industrial processing of potatoes to potato starch the proteins are present in the potato fruit juice. The potatoes are ground and the insoluble material, mainly starch and cell wall material, is removed by hydrocyclones. 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.

2.9 Blood protein

Animal blood, bovine or porcine, is collected at slaughter houses. Blood proteins are used in several applications. Functionality of industrial blood proteins can be grouped in into three categories. First, in the pharmaceutical industry porphyrins are used as raw material. Secondly, in the food industry the functional proteins are used as water binder, fat emulsifier and fresh meat binder. Thirdly, in the feed industry bioactive proteins are used in piglet starter diets. In the pet food/feed industry bulk proteins are used as a protein source. The bulk protein Hemoglobin powder is produced in powder form derived from the blood cells and is highly digestible and the amino acid composition fits with nutritional needs. Hemoglobin powder is used in the food industry as a colouring agent and in the fish, feed and petfood industry as a protein source and appetizer.

3 New protein resources and isolation processes

3.1 Proteins from aquatic biomass

Nowadays, research on aquatic biomass, like micro and macro (sea weed) algae is performed for several end applications, such as biodiesel production.

3.1.1 Microalgae

Microalgae are of main interest because of opportunities to use them for biodiesel production. In order to make the production of biofuel from microalgae cost-efficient, a biorefinery concept for harvesting valuable algae components other than oil is required. Besides oil and an array of secondary metabolites, microalgae contain large amounts of proteins, and to a lesser extent, carbohydrates. The use of proteins from microalgae in food and feed applications not only contributes to supplying an alternative source of proteins but it is also essential to make the cultivation of microalgae for the production of bulk products cost effective. Depending on the techno-functional (physical) and nutritional properties, the microalgae proteins can be used as ingredients for food production.. For the use of food ingredients a high purity of the ingredient is desirable and often a prerequisite. For algae proteins it means the absence of a green color and bitter flavours.

Microalgae have already been long considered as a source of proteins for foods. The reason for this is the high proportion of protein present: The protein concentration based on dry weight can be as high as 47% (w/w), depending on the strain used (Becker 2007). Although analytical methods for determining protein content are available, no extraction methods have been published for the mild, preparative extraction of proteins from microalgae, in which the functional properties of the proteins are retained.

The majority of the research on algal proteins for food application has been focused on amino acid composition and digestibility of the protein fraction extracted from algae (Barbarino, Lourenco et al. `2005). There is presently a lack of information on the techno-functional and nutritional characterization of proteins in microalgae and on the behaviour of these proteins under processing conditions. It is important to device an efficient extraction procedure, without compromising the proteins functionality.

3.1.2 macroalgae (seaweed)

There are three groups of seaweeds; the green weeds, the red weeds and the brown weeds. A study was carried by ECN (Reith, Deurwaarder et al.) to address technological feasibility of seaweed cultivation in the North Sea in combination with offshore wind parks and harvesting and conversion of seaweed biomass to renewable energy carriers and chemicals. Three seaweed species that are native in the North Sea had been selected for potential cultivation: *Ulva sp.* (belonging to the green macroalgae), *Laminaria sp.* (a brown macroalga) and *Palmaria sp.* (a red macroalga). Seaweed biomass in general is a suitable source for a range of CO_2-neutral chemicals, products and secondary energy carriers. The worldwide seaweed industry shows consistent growth. Current applications include the use of fresh seaweed for human consumption (primarily in Asia) and production of phycocolloids

(alginates, agars and carrageenans), feed, fertilizers and extracts for personal care products. Seaweed offers many additional possibilities for production of renewable chemicals and energy carriers for a future "bio-based economy". Seaweeds typically contain a high amount of polysaccharides (approx. 60 w%) that are in principle suitable for production of a range of platform chemicals via fermentation to replace petrochemical bulk chemicals. It is expected that development of hydrolysis and fermentation technology for the specific sugars in seaweeds is possible with the use of modern biochemical and genetic tools. Finally seaweed biomass is a potentially rich source for a range of valuable products including omega fatty acids, colorants and bioactive substances. Several options for energy conversion of seaweed biomass or residues after extraction of specific products or fermentation are considered. The production of bioethanol (through fermentation) has considerable potential in the longer term. Anaerobic digestion for the production of biogas has already shown good results and is therefore feasible in the short term.

Seaweeds, depending on the species, can contain proteins contents between 10 and 30%, and therefore is an interesting source for protein isolation.

3.2 Proteins from plants and leaves

Green plants are fast growing and world-wide available. Therefore, green plants and leaves have a huge potential for the production of proteins. Important crops that can be used for the production of proteins are:

- Grass
- Lucerne
- Alfalfa
- Plant leaves
- Spinach leaves
- Beet leaves
- Jatropha leaves

Leave protein concentrate is obtained by green crop fractionation (Kamm, Gruber et al. 2006). The exploration of green plant leaves can be traced back to the 18th century. At those times it was already proven that protein extracts can be isolated from alfalfa leaves. The leaves are pressed and a protein rich juice is obtained. Upon heating the juice the proteins coagulate and can be removed from the juice. Mild warming yielded a green coloured fraction, further heating delivered an almost colourless precipitate. In the 20th century research on leave protein concentrate focused on use of proteins for human nutrition, because of the widespread availability, high nutritional value, and high protein productivity in green plants.
Of economical interest is a fraction expressed in the chloroplast of green plants, the enzyme ribulose-1,5-biphosphatecarboxylase/oxygenase or rubisco. In spinach leaves, rubisco accounts for 75% of the soluble proteins, in wheat and barley it is 50-75% and in corn 15%.

The first modern industrial process for leave protein extraction was called the Rothamsted process. The procedure based on heat coagulation of green plant juice at 70°C, resulted in leave protein concentrates with 60% 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. Wet fractionation (pressing) leads to two fractions; the press cake and the press juice. The press cake is rich in crude fibre and the press juice contains among others proteins and water-soluble sugars.

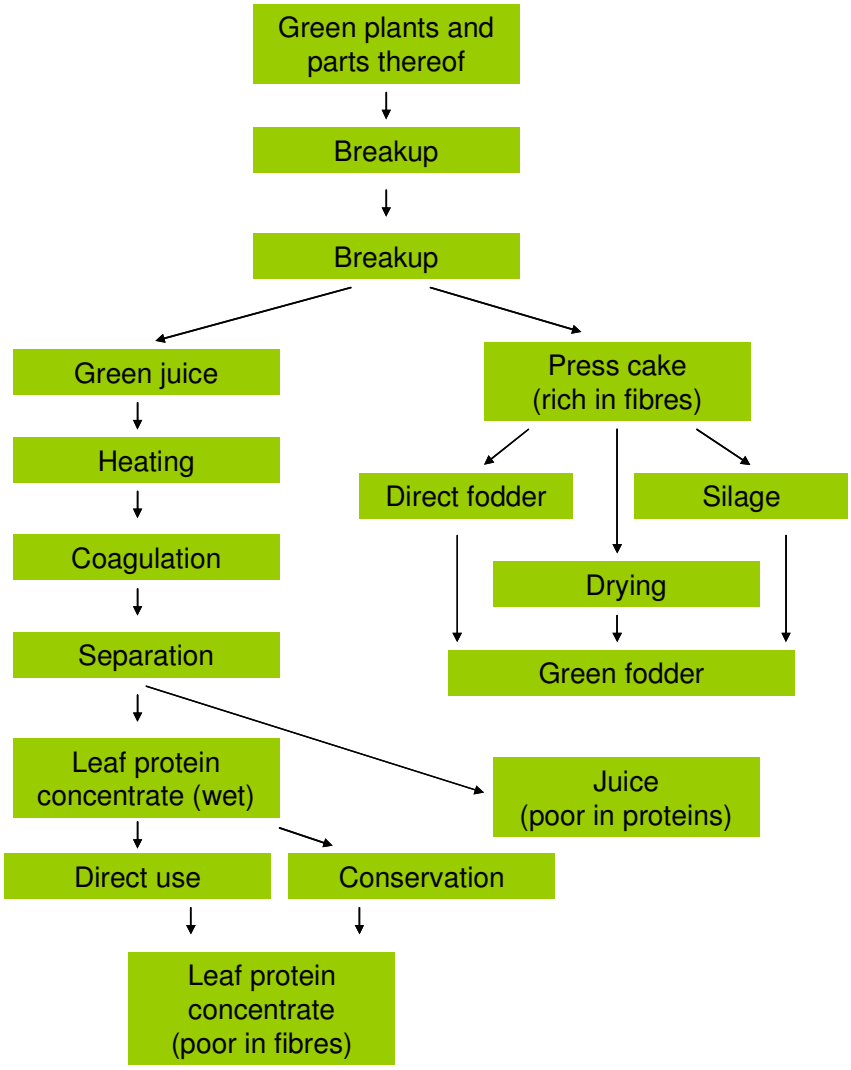


Figure 10. *Flow chart of the fractionation of green plant parts for the extraction of leaf protein*

The company "France Luzerne" has developed a process for the separation of proteins on an industrial scale. The resources, mainly alfalfa, are treated be four main process steps:

- pressing
- heat coagulation
- centrifugation
- drying

In Germany grass is pressed in a centralized factory. The press cake is dried by a traditional grass drying process, while the juice is used to obtain protein by coagulation from the juice that is further concentrated by evaporation. In Austria decentralized pressing of grass is performed to take into account small scale agriculture. The system is built around grass silage fermentation and further processing leads to lactic acid, amino acid mixtures. In Switzerland 2B Biorefineries AG has developed a fractionation system that produces fibre products for isolation applications, protein for feed and bio-electricity. In the Netherlands an existing potato refinery is used as the starting point and by using a refinery that opens up actually all the cells of the grass, an almost quantitative yield of the components in different fractions can be obtained.

Grass and lucerne juice has been fed to animals and it was shown that these leaf proteins could function as an alternative for e.g. fish protein or soybean meal. In feeding (monogastric) animals, the protein content and the amino acid composition is important The amino acid composition of leaf protein concentrates is presented in table 3 (Nasi 1983). Leaf protein concentrate had fairly high contents of lysine and methionine.

Table 3. *Amino acid composition of leaf protein*

Amino acid	**composition (g/6 gN)**
Alanine	6.6
Arginine	3.8
Aspartic acid	7.8
Glutamic acid	9.2
Glycine	5.1
Isoleucine	3.8
Leucine	7.5
Lysine	4.2
Methionine	1.8
Phenylalanine	4.6
Proline	4.9
Serine	3.9
Threonine	3.8
Tyrosine	4.2
Valine	5.2

Jatropha curcas (Lestari, Mulder et al.) is an oilseed crop, which is grown mainly for oil production. Indonesia has a high potential for growing *Jatropha curcas* due to the large land availability. *Jatropha* oil, together with palm oil, will be used as biofuel to provide 5% of the total energy supply in Indonesia by 2025. Considering this, there will be approximately 30 million ton/year of Jatropha seed to produce oil and that will result ca. 20 million ton/year of seed press cake as waste. This waste contains approximately ca. 5 million ton /year of Jatropha protein— a high amount that will be highly profitable to process further into a higher added value product. Besides oil, *Jatropha* seed kernel contains approximately 25-30% protein. After oil removal, proteins will remain in the *Jatropha* cake. *Jatropha* seed protein may have similarities with the other well-known oilseed protein such as soy, Canola, or sunflower protein. In contrast to soy and sunflower, *Jatropha* seed contains toxic compounds such as curcin and phorbol esters, which makes *Jatropha* protein unsuitable for food applications. However, the use of *Jatropha* protein in non-food applications is a potential outlet. Possible non food applications of proteins are in the field of adhesives, coatings, chemicals, fertilizer, such as seed press cake fertilizer and amino acid chelated micronutrient fertilizer.

3.3 Proteins in residues of biofuel industry

3.3.1 Biodiesel

Recovery of oil from fruit or oilseeds is mainly done by pressing (batch wise or nowadays continuously by screw presses). In order to increase the oil yield the seeds can be pretreated by preconditioning. Often the pressing stage is followed by an extraction step with organic solvents, water, or supercritical fluids 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. In table 4 the composition of rapeseed and sunflower is shown (Dominguez, Nunez et al. 1994). The extraction of peptides and amino acids is beneficial since rapeseed and sunflower press cakes contain 40-50% protein.

Table 4. *Composition of rapeseed and sunflower pulp*

	Rapeseed	**Sunflower**
Water	5.0	6.7
Oil	41.6	53.3
Protein	26.2	22.9
Carbohydrates + fibres	23.4	14.0
Ash	3.8	3.1

3.3.1.1 Rapeseed protein

Rapeseed production is the most important EU oilseed followed by sunflower. Soya and olive make up the balance. Rapeseed provides oil (circa 40% by weight) and a residue, the mail (circa 60% by weight). The rapeseed meal consists mainly of proteins (40-45%), fibres and secondary metabolites (e.g. glucosinilates, phenolic compounds). The animal feed market is the main outlet for these co-products.

Cruciferin (12S globulin) and napin (2S albumin) are the major proteins found in rapeseed isolate. Cruciferin has a molecular mass of 300 kDa and is organised in a hexameric structure. Each of the six subunits is composed of two polypeptidic chains (α and β) of about 30 and 20 kDa, linked by a disulfide bond. Napin is smaller, with a molecular weight between 12 and 14 kDa. It is composed of two polypeptides with a molecular mass of approximately 4 and 9 kDa, linked by two interchain disulfide bonds, its compact structure is stabilised by two additional intrachain disulphide bridges

As part of a large cooperative European research programme (ENHANCE: Green chemicals and biopolymers from rapeseed meal with enhanced end-performances), aimed at enlarging the application field of rapeseed components, oil extraction process were adapted to avoid the denaturation of the protein fraction of the grain. A process for the production of isolate from this mildly deoiled meal was developed. This isolate was characterized by a high solubility and very good emulsifying properties.

3.3.1.2 Sunflower protein

In 1985 sunflower seed (Perez 2003) was the fourth major oilseed produced in terns of tonnage (after soybeans, cottonseed, and peanuts) and the fourth major source of edible oil (after soybeans, cottonseed, and rapeseed). Major producing countries are Argentina, EU countries, Russian Federation and other Eastern European countries. Oil and proteins are the main components of the sunflower seed. Sunflower kernels consists of about 20-40% proteins. Globulins constitute most of the sunflower proteins. Globulins are insoluble in water, but soluble in diluted salt solutions. Despite the differences in sunflower protein classification found in literature, it can be concluded that helianthinin, a globulair protein, and sunflower albumins are the major protein fractions in sunflower seeds.

Sunflower meal is obtained as a by-product of the oil extraction process and has a high protein content; about 40-60% depending on the extraction method. This high protein content makes sunflower meal an attractive source for the isolation of proteins. The suitability for food applications depends mainly on the oil extraction method. Due to this process, the proteins may be denaturated to a large extent, resulting in a sunflower meal with a high content of insoluble proteins. Therefore, the main outlet of sunflower proteins is in animal feed. As compared to proteins from legumes and other oilseeds, sunflower proteins have no anti-nutritional components. Sunflower seeds have a high content of phenolic compounds. They attribute to the dark colour of proteinproducts. 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 storage life and stability. Procedures for the removal of phenolic compounds generally alter and/or solubilise proteins, thereby increasing protein losses.

3.3.1.3 Potential protein en amino acid isolation processes

To extract peptides in the form of amino acids from rapeseed and sunflower waste streams, the protein has to be hydrolysed. In principle, proteins can be hydrolysed under acidic or alkaline conditions. Proteins in solution or dispersion can already start hydrolysing under mild conditions, for example at pH8 at 50°C. However, for complete hydrolysis into amino acids more severe conditions are necessary. Complete hydrolysis of proteins into amino acids can be achieved by heating proteins for 24 hours at 110°C in 6 n HCl. But even under such severe conditions, some peptide bonds, like valine-valine and isoleicine-isoleicine, will not be cleaved. Besides this, some amino acids are chemically modified under these circumstances. To prevent oxidation of cysteine into cysteic acid and of methionine into methionine sulfoxide, and the formation chlorotyrosine, acid hydrolysis has to be carried out under vacuum or an inert atmosphere. In contrast, tryptophan is completely destroyed and threonine and serine are partially (5-15%) destroyed.

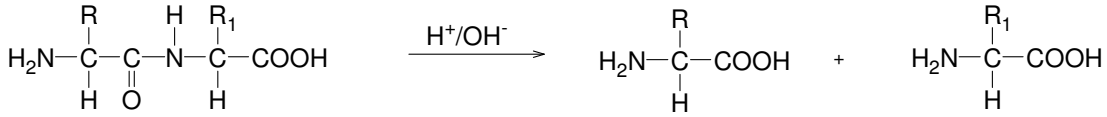


Figure 11. *Cleavage of peptide bond under alkaline or acidic conditions*

Nearly complete hydrolysis under alkaline conditions is possible at 100°C, 5 n NaOH during 18-24 hours. Under these circumstances, tryptophan stays intact, but other amino acids like cystine, cysteine, serine and threonine are destroyed or damaged due to racemisation. In addition, lysine can be transformed in lysoalanine which is not allowed for food applications..

Besides chemical hydrolysis, the molecular weight of proteins can also be decreased by the use of proteases. For example, in the food industry proteolytic hydrolysis is performed to increase the solubility, emulsifying or foaming properties. Basically, enzymes can hydrolyse a protein in two ways; exoenzymes can sequentially remove single amino acids from the end of the protein chain, whereas endoenzymes can rupture the internal bonds in a random manner at any point along the chains. To achieve high degrees of hydrolysis often combinations of exo and endo-proteases are used.

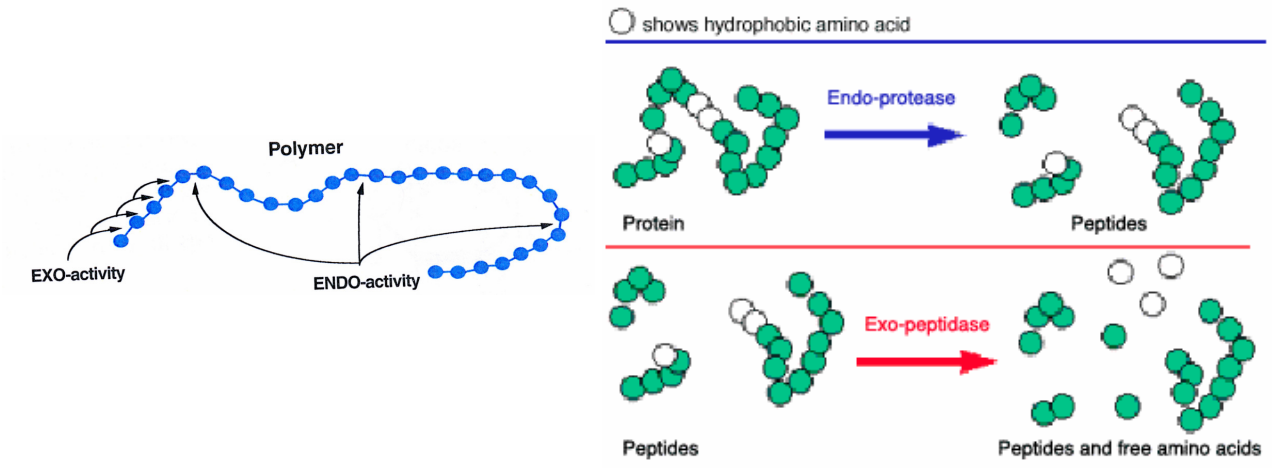


Figure 12. *Endo- and exo- activity of enzymes*

Apart from being an endo- or exo-type, proteases have an additional level of specificity towards certain amino acids. For example, Trypsin is an endo-protease that cleaves only peptide bonds in which an arginine or lysine amino acid residue is involved (Figure 13). This enzyme hydrolyses proteins only at a limited number of sites and therefore has a narrow specificity. However, most of the proteases are less particular about the amino acids encountered and have a broad specificity.

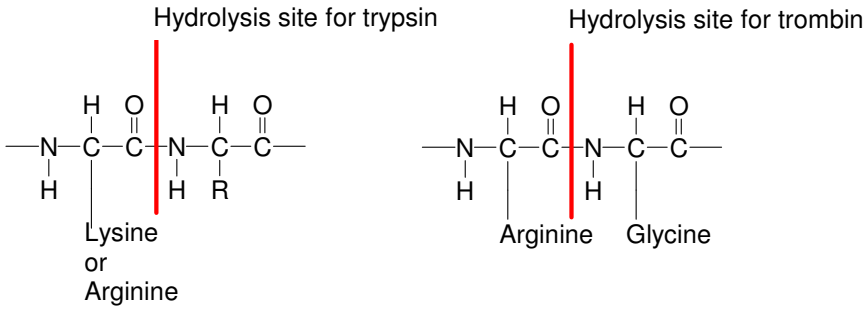


Figure 13. *Specificity of Trypsin and trombin*

To produce hydrolysates with a high degree of hydrolysis, as mentioned, mixtures of different enzymes are used, but also cascade processes, in which addition of series of proteases differing in specificity and optimal conditions. In a patent of Novo Nordisk (Nordisk 1997) it is described that hydrolysates can be prepared having a degree of hydrolysis of up to 90%. However, in this case the dosage of the enzymes is rather high.

With respect to the hydrolysis of proteins to produce amino acids, the chemical pathway is cheaper and economical more feasible. Enzymatic methods may become more attractive in case of breakthroughs, like cheaper production methods or the development of less specific

enzymes. Immobilisation of enzymes might also enhance the economic feasibility. The drawback of hydrolysis under alkaline or acidic conditions is that a lot of salt is being trapped in the amino acids/peptides after recovery. In addition, the severe conditions of the chemical hydrolysis can cause damage of certain amino acids. A non-conventional technique to hydrolyse proteins, by the use of solid acid catalysts is a promising option as it will not require the use of corrosive mineral acids. In this way, less salt is being generated and isolation of the amino acids is facilitated. In order to use solid bases or acids, protein solubility is required. This can be achieved by mild acid, base or enzymatic treatment.

The recovery of amino acids and peptides from by products of the oil production can be performed by different processes and scenario's:
- Extraction of proteins before oil recovery during pre-treatment
- Extraction of amino acids and peptides from the press cake after oil recovery
- Simultaneous extraction of amino acids, peptides and oil.

Extraction before oil recovery
When the main goal is to isolate proteins from a biomass source, proteins can also be extracted before oil recovery. During such a process, the (pre-treated) rapeseed or sunflower oil seeds are enzymatically treated with proteases, or chemically with acids or alkaline solutions in order to hydrolyse the proteins. To obtain optimal hydrolysis the seeds are milled to obtain non-defatted meals. After hydrolysis, both the amino acids/peptides and oil have to be isolated. A complicating factor in this can be the fact that low molecular weight fractions of proteins in general stabilise oil emulsion, which would negatively effect the recovery of the oil. Apart from this, it is also difficult to obtain peptide fractions that are free from oil.

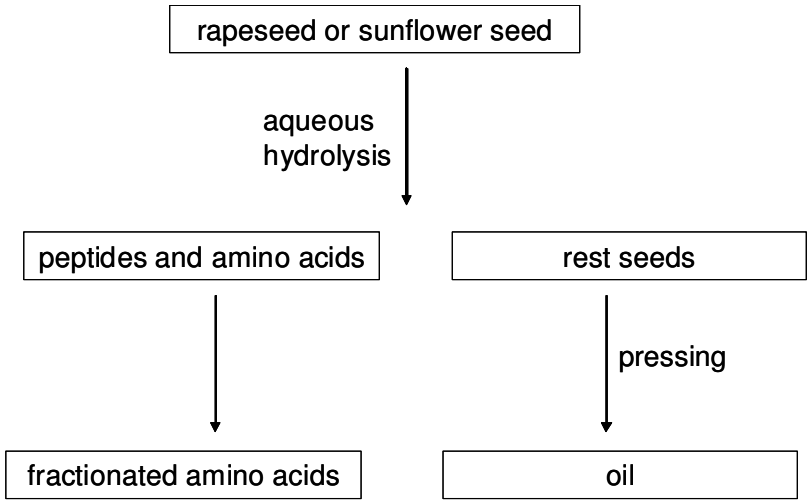


Figure 14. *Production of amino acids from oil seeds*

An alternative way of recovering protein directly from oilseeds is by the use of ultra filtration membranes in combination with reverse osmosis. This process uses less effluent and an increase in isolate yield was shown.

Extraction after oil recovery

Historically, the three most common processes for recovering oil from seeds are hydraulic pressing, the earliest processing method, and the nowadays used (combination of) expeller pressing and solvent extraction. As a solvent, hexane has been proven to be the most efficient and cost-effective means of recovering oil from oilseeds. By performing these methods press cakes are being formed from which peptide and amino acids can be isolated. Since the oil production is usually carried out at elevated temperature and/or in combination with hexane, the proteins will be (partially) damaged. The proteins will be denatured and possibly chemical transformations have been taken place. By this, the hydrolysis of the proteins will be negatively affected and it will be difficult to obtain free amino acids. Therefore, severe enzymatic or chemical hydrolysis processes will be necessary. Another possibility to recover the protein fractions from the dry press cake by means of dry fractionation techniques (milling combined with air classification). By this a fraction is obtained that is relatively rich in protein. From this fraction protein can be recovered by selectively dissolving the protein followed by hydrolysis into amino acids.

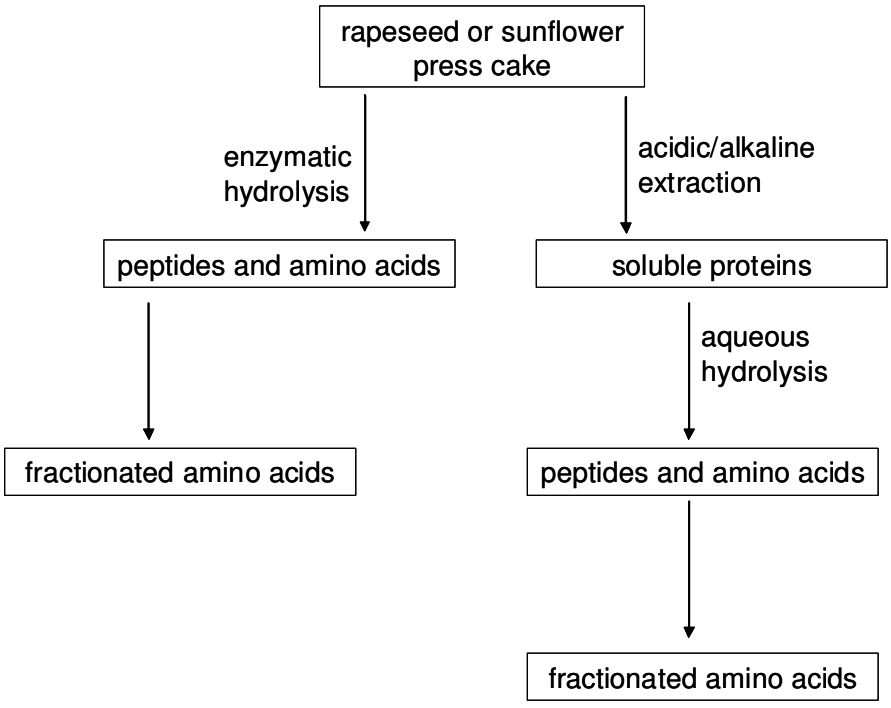


Figure 15. *Production of amino acids from press cakes*

Simultaneous extraction

Due to safety matters and environmental issues the industry is actively looking for alternative processes for hexane extraction. Aqueous extraction processing has gained

considerable interest during the past decade as an environmentally-friendly method for oil extraction (Rosenthal, Pyle et al. 1996). Using this method the oil recovery is 65-75% whereas oil recovery using hexane is higher than 95%. Enzymes can be used to increase the oil recovery. Aqueous enzymatic extraction is a novel technology to simultaneously isolate proteins and oil. The combination of carbohydrases and proteases facilitate the separation of free oil. The general process to obtain protein from oilseeds is depicted in Figure 16.

Critical step that affects oil and protein yield in aqueous extraction is grinding of the starting material. Efficient extraction is possible when cell walls are broken down and small particles are formed. Regarding the extraction step itself, the ratio between the solid particles and liquid, pH, time, temperature, energy of agitation and the number of stages are important factors. In general, both high oil and protein yields are obtained when the extraction is performed not near the isoelectric point. Under acidic conditions protein concentrate is produced whereas under alkaline conditions isolates are obtained. Optimum pH conditions for oil and protein extraction of sunflower and rapeseed are respectively about pH10 and pH6.6. Protein yields in these cases are about 90%. Use of enzymes in general increase the oil and peptides/amino acids recovery. In respect to this, enzyme mixtures (pectinases, cellulases, proteases) with combined activity give the best results. Aqueous extraction in comparison to solvent extraction has an advantage in the way that higher quality of the protein is obtained

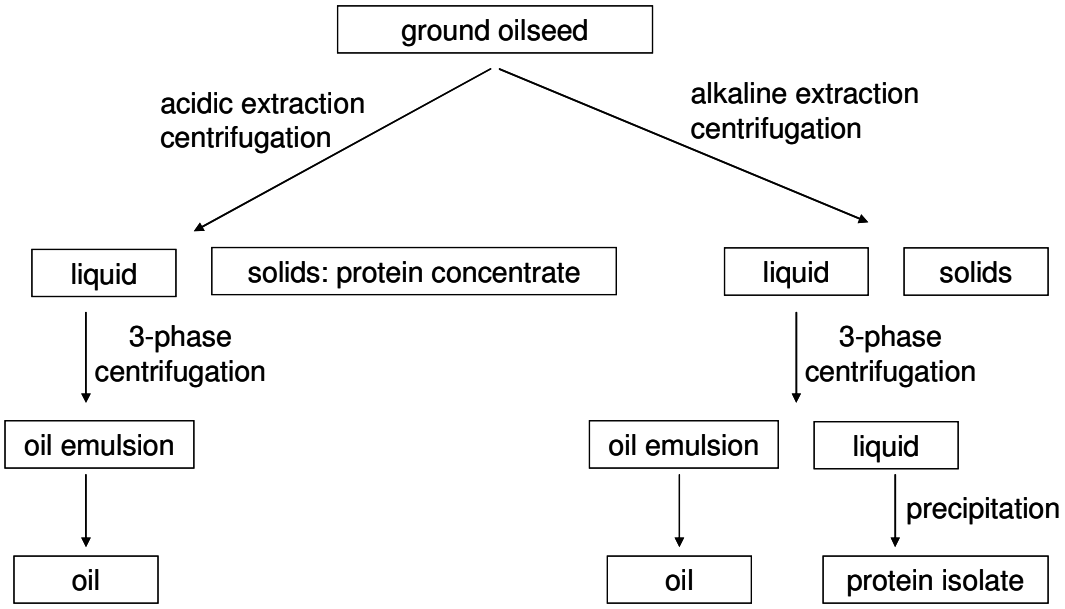


Figure 16. *Simplified process of protein recovery*

To isolate protein after the extraction usually demulsification is needed and centrifuges are used

to obtain highest oil and recovery. After centrifugation usually three layers are formed:

- oil layer
- water layer
- solid phase

Protein can be recovered from the aqueous phase and/or the solids. After the separation step, most of the proteins may be recovered as concentrate in the solid phase or as an isolate in the aqueous phase, depending on the pH of the extraction. When the extraction took place under alkaline conditions, protein is obtained by iso-electric precipitation. Despite the fact that the use of hazardous solvents are avoided, this method also has several drawbacks. Oil yields are still low compared to hexane extraction and therefore the residue and protein fraction can still contain a considerable amount of oil.

In several papers Zhang et al investigated the aqueous extraction of rapeseed (Zhang 2007; Zhang, Wang et al. 2007). After wet milling, the slurry was treated with a combination of cell-wall degrading enzymes pectinase, cellulase, and β-glucanase (4:1:1, v/v/v) at a concentration of 2.5% (v/w) for 4 hours. Alkaline extraction was performed at pH10 during 30 minutes, followed by hydrolysis of the protein using Alcalase (1.5%) at pH9 during 3 hours. After centrifugation and down stream processing the free oil and the aqueous phase, containing the hydrolysates, were obtained; yields for oil and protein were 75% and 80% respectively. Protein hydrolysates were composed of about 96% of peptides with a molecular weight less than 1500 Da, of which approximately 87% were less than 600 Da. The protein hydrolysates were purified using a macro porous adsorption resin.

Protein isolate from defatted sunflower was subjected to hydrolysis using and endo-protease, Alcalase, and an exo-protease, Flavourzyme resulting in a hydrolysate with a degree of hydrolysis of about 50%(Ordonez, Benitez et al. 2008). Another study was performed on the defatted oil seed sunflower wholemeal. In this case also an combination of Alcalase and Flavourzyme was used for protein hydrolysis. The highest degree of hydrolysis that was achieved was 40%. Apart from the degree of hydrolysis also the amount of free amino acids was measured. It was revealed that the free amino acid found in the highest proportion in the hydrolysate was aspartic acid, which accounted for over 50% of the free amino acids present.

Hydrolysis of proteins in by products of oil production, results in complex mixtures containing many different compounds. To extract peptides and amino acids from these mixtures, reactive extraction, electrodialysis and ion exchange chromatography can be used. The separation of a mixture of amino acids into pure fractions can be achieved by selectively modifying one (or more) of the amino acids. Based on the modification process the isolation technique can be selected. For example, when the charge of certain amino acids is changed, electrodialysis could be an option.

3.3.2 Bioethanol

Converting cereal-based crops, like corn and wheat, into ethanol using first generation biofuel technology yields large quantities of a by-product called distillers grains, for only the simple carbohydrate portion (starch contained in the seeds) is used as a source for ethanol

fermentation. On an average dry weight basis, the corn kernel contains about 70% starch and the wheat grain contains 55% starch (Duke). Typically first generation ethanol fermentations have a 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, leaving a low solid containing liquid bottom stream. The following figure shows schematically the basic process steps involved in preparing dried distillers grains with solubles after separation of the ethanol product.

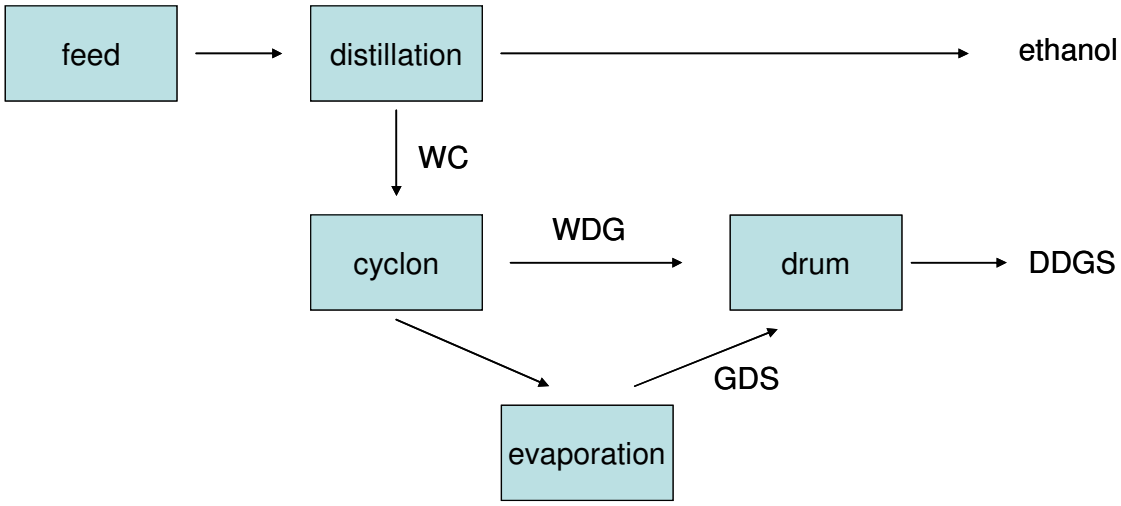


Figure 17. *Simplified process of bioethanol production and DDGS formation*

The fresh distillers grains (WC) 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%, thus producing dried distillers grains with solubles (DDGS). In the system there are four basic and intrinsically different streams:

1. WC : Fresh Wet Distillers Grains with Solubles (Wet Cake)
2. CDS: Condensed Distillers Solubles
3. WDG: Wet Distillers Grains
4. DDGS: Dried Distillers Grains with Solubles

The chemical composition of the four distillers grains streams are quite different, although on paper the only difference between the WC and DDGS is the moisture content. But in fact, the induced heat treatment changes the chemical structure of the material as can be visually

seen with the DDGS being significantly darker in colour. The series of heating steps negatively affects the physical protein properties, resulting for example in a lower solubility.

The current majority of ethanol technological research directions is focused on converting the complex carbohydrates portion of lignocellulistic feedstocks. Also known as waste agricultural streams, lignocellulistic feedstocks, like wheat straw or stover from corn and rapeseed, have a huge potential to produce extra ethanol at a lower associated cost and energy demand. Corn stover consists of the leaves and stalks of maize plants left in the field after harvesting. The agricultural uses of rapeseed cultivation are currently limited to the oilseed propagation for its fatty acid content. The pods, stem and leaves all form the residual agricultural waste known as stover.

Table 5. *Protein contents in different feedstocks and their DDGS*

Feedstock	**Protein content [%]**
wheat distillers grain	38
wheat straw	7
wheat straw distillers grain	18
rapeseed cake	43
rapeseed stover	26
rapeseed distillers grains	55
corn	10
corn stover	3
corn distillers grain	30

After a great deal of the carbohydrates have been converted to and recovered as ethanol, the biofuel side stream feedstocks 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 feedstocks are so high in protein content that they could be used directly as animal feed and marketed for their relative protein content. These protein contents can be compared with pure corn gluten meal which due to its high protein content of typically 60% sells in the premium animal feed price range of 300 – 350€/ton. However the ash, other component impurities and possible protein structure alteration of the proteins in DDGS may result in a lower digestive value. A feasible option would be to remove and isolate the large protein and/or amino acid content in a pure form. Separation of proteins from biomass feedstocks is a new and upcoming field of research. One good example and potentially feasible technology is the use of proteases to solubilize and separate the proteins. In addition to solubilizing the protein, protease is able to digest the proteins to a high degree into their free amino acids. And considering that each free amino acid possesses a unique physiochemical property the separation and isolation should be relatively straightforward, granted the technology is in its infancy. Electro-dialysis and advanced chromatography are two proposed process technologies that could be employed industrially.

3.4 Miscellaneous

In principle, all oil seeds (and fruits) contain storage proteins. Apart from the seeds that already have been mentioned, also cottonseeds, olive, crambeseeds, linseeds etc. possess proteins. These products are of less relevance and therefore shortly discussed.

Olive protein
Proteins, both from animal and plant origin, are valuable compounds that play an important role in human nutrition. Therefore, the search for new sources from which proteins can be extracted is actual. Olives are mainly used for the production of olive oil. Although olive pulp, after oil removal, contains a significant amount of protein, olive protein is not commercially produced. Therefore, it is interesting to exploit the possibilities of extracting protein from olive pulp after the extra virgine oil is removed. Side streams of the oil production process could be interesting to be used as a source for olive protein.

Crambe proteins
Several new oil crops, such as Crambe abyssinica (Massoura, Vereijken et al. 1998), have been considered for the production of oil. The crude protein content is about 20% in the whole seed and reaches up to 50% in the meal remaining after dehullling and defatting. Crambe protein has a well balanced amino acid composition, and therefore is of interest for the feed sector.

Finally, some minor products, which will not be further described, contain proteins:
- coffee pulp
- dug wheat
- shrimp meal
- hemp

4 Feed markets

Feed include two main categories: Compound feed, which is mainly produced in industrial operations, and other feeds, mainly roughage but also simple concentrates and raw material which are directly used on-farm. Reliable balance data for compound feed from industrial origin are available, whereas for roughages, particularly permanent pastures and meadows, reliable data can only gathered for area, not for production.

The average annual production of compound feed in EU25 between 2003 and 2007 was 144 million tonnes. This large production of compound feed is mostly for consumption within the EU, since exports to third countries are less than 1.1 million tonnes. To meet their needs, feed industries in EU must import annually 34 Mt of raw materials, mainly, by-products of primary industrial transformation of different food commodities (26-27 Mt of oilseed cakes, 3.0-3.5 Mt of CGM and DDG, 1.5-2.0 Mt of molasses and 1.4-1.8 Mt of beet pulp). Although, overall, the EU is self sufficient for cereals, 5.0-10 Mt of imported cereal grains are used by the feed industry annually. The most remarkable deficiency in feed ingredients in EU are protein concentrates, which, except for pulses and dried forages, are produced domestically below 70% of our needs .

Animal feed used 158 Mt of cereals annually in 2005-2007, one third of it through direct farm use and 48% as compound feed ingredient. Common wheat, maize and barley are the most important, adding to 80% of the total used for feed. The projected increase in use of cereals for feed by 2014 amounts to 10 Mt (accounted mostly by wheat – 6.7 Mt – and maize – 6.1 Mt- with minor reductions in other coarse grains).

The average annual use of dry pulses for animal feed was 3.8 Mt (2003-2005), with more than half of it being peas (2.4 Mt). Overall, more than 75 % of pulses are utilized as animal feed with self sufficiency ranging between 79 and 120 %.

The feed industry absorbs the totality of oilcakes produced domestically (15-20Mt from both domestic and imported seed) as well as imported cake from third countries (an average of 18-20 Mt). The self sufficiency for oil cakes is 45-47 %, however, this estimate accounts as a domestically available resource the cakes obtained by EU crushing industries from imported soybeans (around 14 Mt/year).

The most significant oilseeds are soy and rape; in Mediterranean countries, olive is also a mayor source of fat. Part of the available oilseeds, particularly soy and rape (1.5-2.0 Mt) are used as full-fat supplements for animal feeding, either raw or heat/mechanically processed, but the vast majority is processed for oil extraction. Fats are also added to animal feed in the form of fats and oils, the majority of them being imported palm products (0.25-0.28 Mt/year) and soy or rape oils (0.10 Mt each).

Among the remaining common feed ingredients produced in the EU territories, potatoes and their derivatives are the main amylaceous material. The EU is self sufficient for potatoes, 60% which are marketed as a fresh vegetables and only 15-20% (4-11 Mt) are used for animal

feeding in different forms. The remainder 20-25% is either processed to various food products, industry transformed for starch and alcohol or used as seed.

Other industrial by-products used for animal feed are those from milling, brewing, distilling and starch industries. In 2005, the amounts of milling residues were 0.38 Mt for maize, 0.40 for rye and 0.44 Mt for other cereals and 8.16 Mt for brewing, distilling and starch. Similarly, vegetable by-products and waste for animal consumption, mainly produced by the food industry, provides annually 1.3-1.7 Mt of fresh material with varying dry matter content.

Finally, a whole range of vegetable materials exclusively for animal feeding are summarized in the Crops supply balance sheets. The fodder products are divided into two main groups: fodder from arable land, be it annual or perennial, and fodder from permanent meadows and grasslands, as well as other minor permanent crops. The interpretation of data in these categories is difficult. On the one hand, surveys to gather the data are probably less systematic than for other crops, since fodder in these groups are in some cases harvested and consumed in the same farm operation without reliable recordings on yields, while others are grazed. On the other hand, the products included in these categories, which are reported as green fodder production, cover a wide range of dry matter contents, making it very difficult to estimate the average energy content of the crops. These difficulties might be the reason why reporting by the different countries is not as systematic as for other crops, and there are several categories with data for only 5 or 6 countries.

5 Chemicals from proteins and amino acids

For the production of chemicals functionalised with a nitrogen group the basis building block is anhydrous ammonia. The potential of chemicals that can be produced from proteins is dependent on the amount of bound nitrogen in the base chemicals used for production of polymers and resins

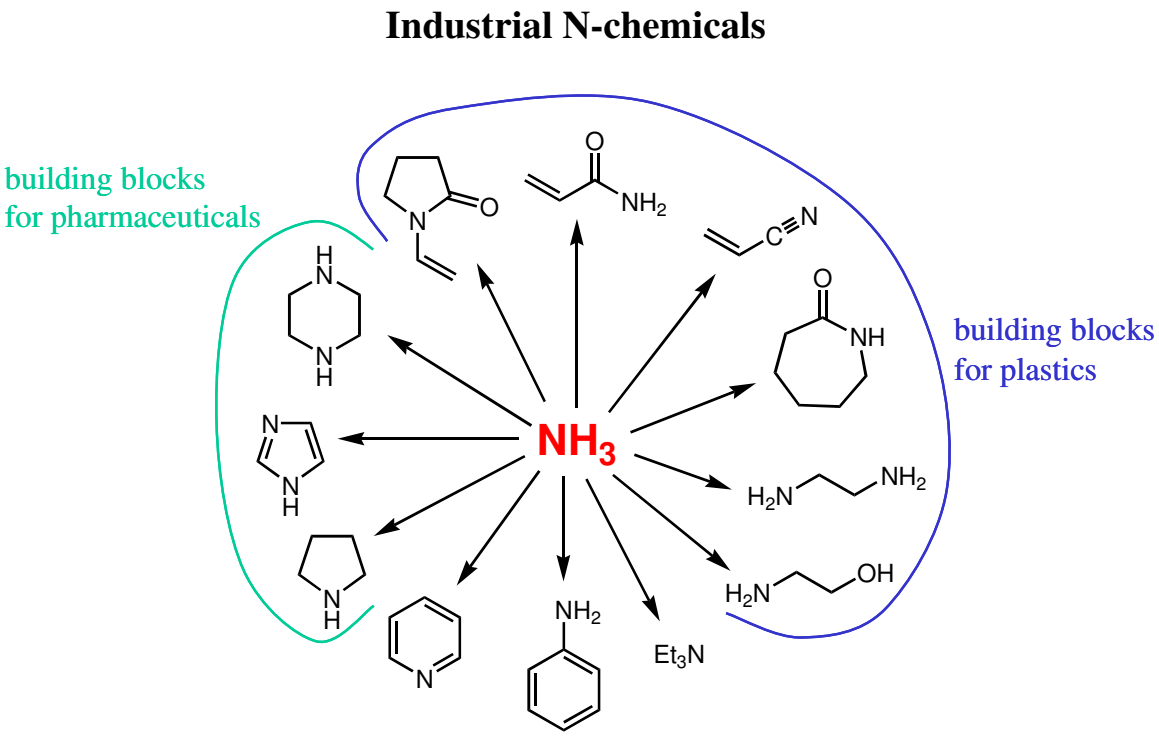


Figure 18. *Potential of industrially produced N containing chemicals that can be replaced by chemicals derived from proteins and amino acids*

Traditionally, based on their nutritional value, taste, physiological and chemical characteristics, amino acids are being used in human food and animal feed (Leuchtenberger 1996). Hydrolysis of proteins, mainly vegetable, into peptide and amino acids is performed on industrial scale to produce food ingredients with savoury, meat like flavour. Monosodium glutamate (MSG) has been used for a long time as a flavouring enhancer and is the amino acid with the largest production capacity. In food, also glycine, cysteine and oligopeptides are being used. Essential amino acids, like lysine, methionine, tryptophan and

threonine are added to animal feed. Last decades, amino acids gained interest in pharmaceutical, cosmetic and chemical industry

Amino acids can be produced chemically, via enzymatic catalysis, by fermentation and by extraction from protein rich materials (Leuchtenberger, Huthmacher et al. 2005). The two biotechnological processes, fermentation and enzymatic catalysis, are most exploited. Extraction of amino acids is at this moment not suitable for large-scale production. However, due to the increasing amounts of biomass waste streams, the extraction from these materials will become more and more important in the future. Therefore, also the search for other applications like chemicals will become more relevant.

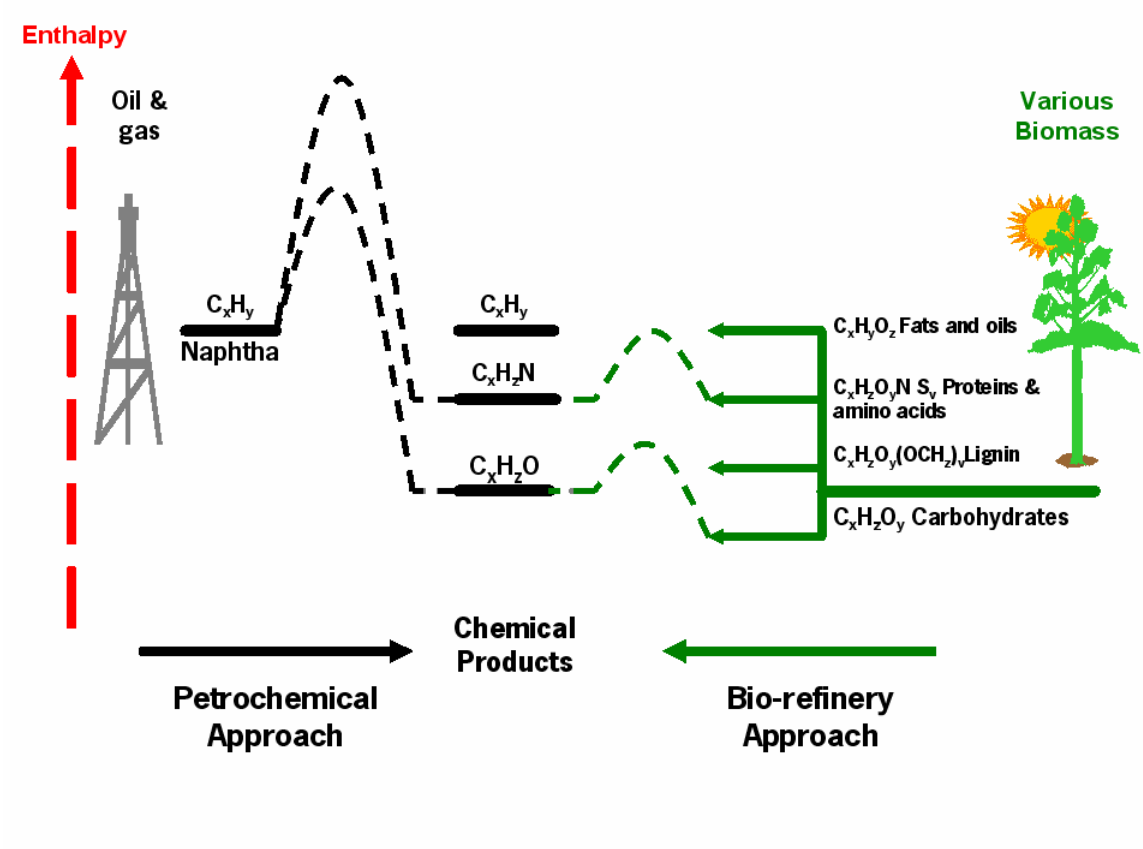


Figure 19. *The use of biomass as a more energy-efficient raw material.*

The potential of biomass as a feedstock for energy supply or as a feedstock for chemical building blocks is recently obtaining much attention, which is e.g. reflected by the publication of various roadmaps describing the (general) potential of biomass as a feedstock in the coming decades. Biomass consists of lignocellulosic and related materials (70-75% of biomass), oils and fatty acids (15-20%) and proteins (about 5%). These compounds comprise carbon, hydrogen, oxygen and nitrogen as elements. This implies that in principle almost all

of todays' important organic bulk chemicals can be completely or to a very significant extent be based on biomass. In 2004 in the US, the Pacific Northwest National Laboratory and the National Renewable Energy Laboratory published a roadmap study "Top Value Added Chemicals from Biomass" (Werpy and G. 2004). From an initial list of more than 300 candidates, based on criteria such as current state of the art, known market data, properties and performance, this list was narrowed down to 12 *chemical building blocks* that can be derived from sugars and derivatives. As these are all derived from easily accessible carbohydrate resources (e.g. wheat, maize, potato, sugar beet) the perspectives for these building blocks will be similar in Europe compared to the U.S. These platform chemicals are used to produce all the major bulk chemicals. The majority of the bulk chemicals can be produced based on 6 platform chemicals (ethylene, propylene, C4-olefins, benzene, toluene and xylene). On the short to medium term it may be expected that the chemical industry is more prone to adapt existing bulk chemicals based on biomass than new bulk chemicals based on biomass, having a unique structure, different than todays' chemical building blocks.

Traditionally, the chemical industry is using fossil feedstocks for the production of chemicals. Due to the decrease in availability of fossil feedstocks and environmental issues, biomass is becoming an important raw material for the production of chemical compounds. Apart form these aspects, the use of biomass can have also advantages in the synthetic pathways. The petrochemical industry uses simple molecules, like ethylene, to produce functionalised chemicals for which co-reagents, catalysts and many processing steps are used. Therefore, it is more efficient to make functionalised chemicals starting from materials, like amino acids, that already possesses functional groups (Scott, Peter et al. 2007). In figure 19 this is schematically depicted.

In the literature chemical reactions in which amino acids are involved are diverse. Most of the reactions focus on the transformation of amino and carboxylic acid groups, for example the decarboxylation of α-amino acids. In table 6 chemical compounds that are derived from amino acids are given.

The conversion of lysine and phenylalanine in respectively ε-caprolactam and styrene is of much relevance for the chemical industry since these compounds are industrially produced on a bulk scale.

At this moment the processing of different biomasses, like rapeseed and sunflower seeds, is mainly related to the feed and food area. However, it is expected that within a couple of years several biomass related raw materials will be manufactured. The chemical industry can use these products as starting materials for the production of their polymers etc.

Table 6. *Chemicals derived from amino acids*

Amino acid	⟶	**Chemical**
Alanine		Ethylamine
Asparagine/Aspartic acid		2-amino-1,4-butanediol, Amine tetrahydrofuran
Glycine		Nitric acid, oxalic acid
Leucine		Isoprene
Lysine		ε-caprolactam
Phenylalanine		Cinnamic acid, Styrene
Proline		Pyrrolidone
Serine		Ethanolamine
Threonine		Isopropanol amine
Tryptophan		Catechol, Muconic acid
Tyrosine		p-hydroxy-cinnamic acid
Valine		Isobutyraldehyde

6 Technical applications of proteins

The main outlet for proteins is the food sector, however, proteins have also potential to be used in technical applications.

In the past proteins were commonly used in non food applications. Henry Ford was an important early pioneer in soy protein utilization, applying these technologies to improve his automobiles. Products such as plastics were developed. Regenerated protein fibers have been made commercially since the 1930s. Several have been based on casein and soy protein, the fibers being produced by wet spinning. In the paper industry, proteins have been used as sizing agents, binders, and adhesives. Glue, derived from collagen, was the first material used for bonding paper and as an adhesive in paper coatings. Today, the use of glue is confined to specialty applications in paper converting. In the late 1800s, when the demand for coated paper for the halftone printing process increased, casein rapidly replaced the collagen-based glues. Soy protein, a protein with comparable properties as casein, also has been used as a binder in coated papers. In addition, plywood adhesives based on soy protein were developed. In the recent past gelatin was used for more than 100 years as a binder in photographic products. In the early 1900s, research demonstrated potential industrial applications of soy protein in both plastics and adhesives. In the last few decades, hydrolysates from keratin, gelatin, and wheat gluten have been used in cosmetics, e.g., as surfactants in shampoos.

Table 7. *Non food applications of industrial proteins*

Products	**Protein**	**Example**	**property**
adhesive	casein, wheat gluten, soy protein, gelatin	water based hot melt	processing tack bond strength
coating	soy protein, casein, zein	paint ink paper/packaging coating	film forming properties strength water resistance
surfactans	keratin, wheat gluten	emulsifier detergent wetting agent	surface tension stabilisation of interface
plastic	soy protein	packaging	melt strength tensile strength water esistance

When developing technical applications based on industrial proteins, specific properties should be considered depending on the target application. Examples are adhesion and bond strength for adhesives, resistance against water for coatings, and strength for plastic materials. Table 7 gives an overview of a number of technical applications of proteins and the requirements and respective routes to obtain good product performance.

As a result of the rise of petrochemicals in the 1960s, proteins were replaced by synthetic polymers in the non-food sector. An important reason for this substitution has been the lower price of petrochemicals, but differences in performance also have been important in this respect. However, during the last decades, there was an increasing demand from consumers and industries to replace synthetic polymers with polymers from renewable resources. In addition to this, as described in chapter 3, new streams of biomass that contain significant amounts of proteins will become available in the near future due to biorefinery processes. In some cases like Jatropha protein, that is not suitable for food consumption, have possibilities in the non food sector.

7 Protein value chains

7.1 Potential economical value of isolation of proteins from biomass streams

In Europe a number of crops are being grown with significant protein contents. In table 8 the amount of "protein" that is produced in Europe is depicted (Prolea 2008).

Table 8. *The production of protein rich materials in EU27*

	Production (1000 tons)	Protein (1000 tons)
Soy grain	1229	467
Sunflower seeds	6338	1022
Rapeseed	16125	3225
Pea	2910	640
Fodder	3828	727
Corn gluten feed	2311	485

The world production of vegetable oil has doubled since 1986 and reached a volume of 130 Mt in 2007. Palm and soy oil have the lead, each taking 30% of the volume, followed by rapeseed with 15% and sunflower with 9% of the volume. In Europe, more than 90% of the soy grains are imported. The production and consumption of olive oil is mainly restricted to Europe.

Table 9 *Production volumes of oils*

	World production (1000 tons)	EU production (1000 tons)
Soy	37842	2707
Palm	38310	-
Rape	18720	6953
Sunflower	10890	2128
Arachide	4220	-
Cotton	5060	-
Olive	3024	2258

Since the production of oil increased, also the amount of press cakes, which contain high amounts of protein increased (Table 10)

Table 10. *Production of press cakes*

	World production (1000 tons)	EU production (1000 tons)
Soy	15887	11543
Rape	27809	9855
Sunflower	12272	2622

Besides press cakes from the oil production, also other protein rich materials are currently being produced on commercial scale. The main outlet for these materials at the moment is as animal feed. Prices of these products are given in Table 11 (Ladepeche 2010).

Table 11. *Prices of protein rich materials*

Product	**Price (€/ton)**
Soy grain	410
Soy cake	300
Rapeseed	300
Rapeseed cake	150
Corn	120
Corn gluten feed	120
Sunflower	330
Sunflower cake	135
Bread wheat	100
Durum wheat	140
Barley	90
Lucerne	120
Beet pulp	90
Wheat straw	40
DDG (wheat)	145
Fish meal	140
Oil (rape, soy, palm, sunflower)	900

The most valuable compounds in these biomass streams are the proteins. It can be expected that the economical value of these biomass streams can increase significantly when the proteins are being isolated. A number of proteins have been produced commercially for decades. An estimate of the prices of these proteins are depicted in the table. In general, it can be stated that the prices of the pure proteins are much higher than the biomasses containing these proteins. Proteins that are not being denatured during the isolation process like casein, soy protein and pea protein have a higher economical value than proteins that are denatured, like maize gluten and potato protein.

Table 12. *Prices and availability of commercially produced proteins.*

Source	Protein	Price [€/ton]
potatoes	tubertin	1000
cereals	wheat gluten, barley	1000-2000
soybeans	soy proteins	2000 (concentrate) 3000 (isolate)
milk	casein, whey	5000 (whey concentrate) 10000 (whey isolate) 4000 (casein)
blood meal	haemoglobine	400
meat, bones, hides	collagen, gelatine	1000-5000 (gelatine)
hair, wool, feather	keratin	300 (feather meal)
maize	maize gluten, zeine	300 (gluten) 25000 (zein)
peas	pea protein	3000

Based on this, the development of biorefinery concepts, to isolate proteins from biomass streams is an interesting option from economical point of view.

7.2 Economical value of proteins from agro-side streams

Several primer side streams of the agro industry contain protein. Usually, they are only used to enrich the soil. The protein content of these side streams is often low, but when this protein can be economically collected, a big amount of protein becomes available for animal feed and/or chemistry.

In table 13 several biomasses are classified according to their protein content. The group with a lot of protein, like soy cake, has many possible applications with a high value, usually as animal feed. The second group with a protein content of about 25-45% is already less attractive as animal feed. The economical value depends on the protein content, but also on the presence of sugars that can be of beneficial for pigs. Besides this, the presence of anti nutritional components can play a role in the determination of the price. The third group has a protein content of 10-20%. Usually, this type of materials does not have high value and therefore is left on the field. The same counts for the fourth group with a protein content of 5-10% and the fifth group that almost does not contain any protein. 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.

Table 13. *Opportunities of new protein sources*

	Examples	**State of the art**	**Opportunities**
Group 1 protein content >50%	soymeal rapemeal meat meal	already applied due to high protein value	for toxic materials like Jatropha for diluted streams
Group 2 protein content 25-40%	press cake rapeseed sunflower seed meal slaughterhouse waste	already applied (middle value, pigs)	for toxic materials
Group 3 protein content 15%	rapeseed hulls soy bean pods beet leaves	applied but low value	separate protein
Group 4 protein content 5%	fresh grass rape straw soy straw corn stover sunflower seed hulls	primary residu	when logistics costs are low and relative small scale
Group 5 no protein	wheat straw	primary residu	when logistics costs are low and relative small scale

When technologies will be developed that both can isolate (hemi)cellulose and protein, new protein containing biomasses will become available that are relatively cheap. This is attractive because amino acids have more value than sugars and therefore making such a technology economical feasible.

7.3 Current technical applications from proteins

About 0.3 Mton of proteins are used in glues and other applications (see Table 14), mainly from animal origin like gelatines or caseins (Bos, Elbersen et al. 2010) . An important application area for proteins are 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 find still limited applications in the non-feed, non-food markets.

Table 14. *Non-food applications of proteins (according to Eurostat)*

Products	**Volume actually non-food (tons)**
Wheat gluten (excluding wheat gluten prepared for use as a glue or as a glazing or dressing for the textile industry)	141806
Peptones and their derivitives; other proteins substances and their derivitives; hide powder including glutelins and prolamins; globulins; glycinin; keratins; nucleoproteids; protein isolates	34020
Casein glues	18623
Caseinates and other casein derivatives (excluding casein glues)	35977
Gelatin and its derivatives	97139
Bone glues; other glues of animal origan (excluding casein glues)	14508
Total	**342.073**

7.4 Chemicals and protein availability

The amount of chemicals that can potentially be produced from proteins is estimated by estimating the amount of bound nitrogen in the base chemicals used for production of polymers and resins. A minimum of 10 Mton of chemicals might be produced from proteins. The weight of nitrogen bound is circa 2.3 Mton, taking into account a mean nitrogen content in proteins of 16 %, a total of 14 Mton of proteins would be needed.

Table 15. *Volumes of chemicals that can be derived from proteins (according Eurostat)*

Products	**Volume possibly bio-based (tons**
Methylamine; di- or trimethylamine and their salts	352224
Diethylamine and its salts	23645
Ethylenediamine and its salts	170267
Hexamethylediamine and its salts	792660
Amino alcohols; their ethers and esters with only 1 oxygen function and their salts excluding monoethanolamine and its salts; diethanolamine and its salts; triethanolamine and its salts	152283
Acrylonitrile	727435
Melamine	396804
6-Hexanelactam (epsilon caprolactam)	1928826
Urea resins and thiorea resins; in primary forms	4183224
Isocyanates	1631864
Total	10.359.233

For utilizing proteins for production of chemicals, to reduce fossil energy use and greenhouse effect, it is worth looking at protein sources that are at the moment unused or underutilized. Cases were protein is underutilized are:

- Burning of animal proteins because of hygiene measures (BSE). We estimate that in the EU some 1 million tons of animal protein is burned (for energy) which could also be used for chemicals if proteins would be de-polymerized into amino acids adequately. The cost for these amino acids would essentially be the energy content, which is much lower than the energy cost of N-fixation.

– In intensive animal production systems protein supply is higher than required. This is explained by the fact that the amino acid supply in feed does not mach the amino acid profile needed by animals. Animals require a minimum of 9 essential amino acids. An oversupply of protein in feed is used for energy by the animal and N is excreted. Though fixed N is excreted (as urea and manure) by animals and partially used as fertilizer much is volatilized, leached and denitrified. The high levels of volatilization, leaching and denitrification in intensive animal production systems constitute a problem. Manure disposal is costly in intensive animal production systems in the EU. This means that it would be attractive to decrease the protein intake of animals. On top of this the value of protein as a chemical is many times higher that the equivalent value as fertilizer. We estimate that at least 25 % of the protein in animal feed (in intensive animal systems) could be reduced while maintaining the essential amino acids in the feed. This requires biorefinery of the feed to obtain a lower protein feed containing the essential amino acids (and other characteristics). Non-essential amino acids could then used for production of N containing chemicals.

In figure 20 the share of protein use in the EU-15 is shown. Protein rich feeds accounted for 21 million tons of protein in 1999. In the 2005 protein rich feeds accounted for 23.5 million

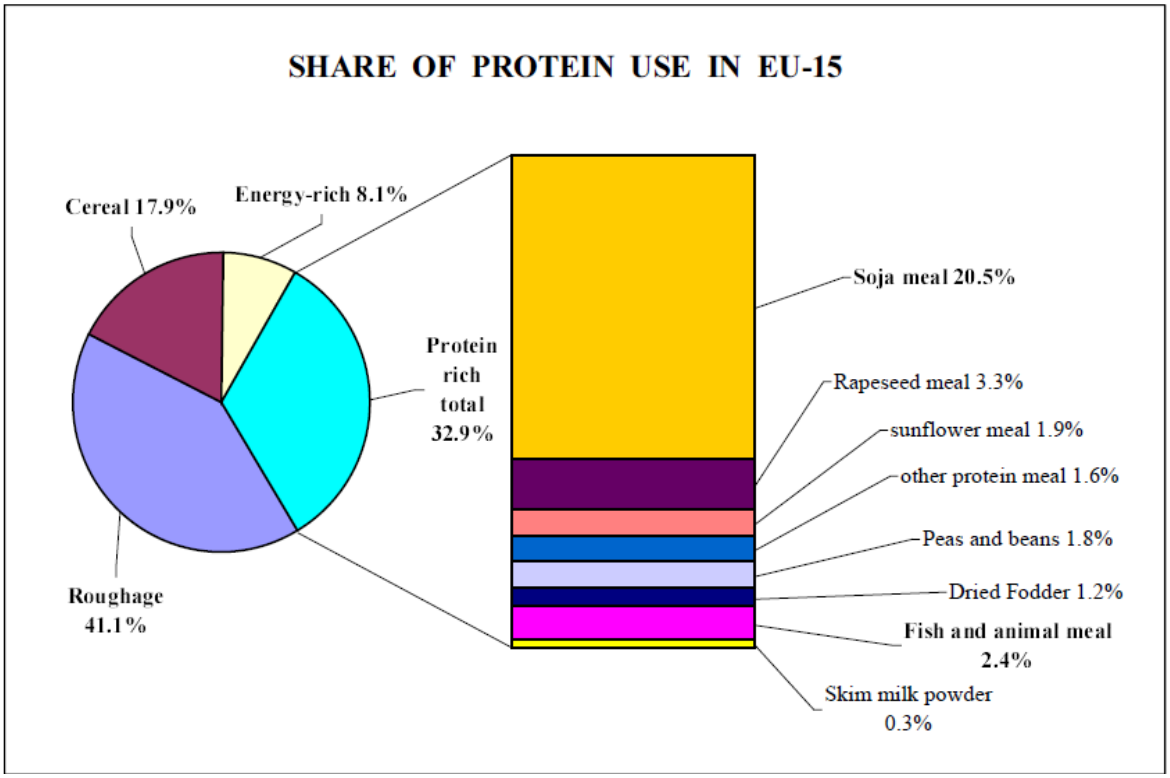


Figure 20. *Share of crude protein in total animal feed in the EU-15 in 2001 based on a total protein use of 64 million tons*

tons of crude protein in the EU-25.

Obvious targets of biorefinery (of proteins and amino acids) would be:

- 50 million ha of intensive grazing land in the EU producing 500 million tons DM of grass. Which contains at least 15% protein = 75 million tons of protein. Protein levels in the grass are much higher than required by cattle. Biorefinery of grass and utilization of protein for other animals (pigs or poultry) or chemicals is then possible. Biorefinery of 20% of the grass would yield 25% amino acids for chemicals = 3.75 million tons of protein (for chemicals).
- High protein meals used mainly for animal feed (Copra, Cottonseed, Fish, Palm Kernel, Peanut, Rapeseed, Soybean, and Sunflower Meal). In 2008 the EU produced some 25.7 million tons per year and consumed 52.6 million tons. If we assume a 50% protein content in meals this would mean that the EU consumes some 25 million tons. Refining the material could yield (25 x 0.25) 6.25 million tons of amino acids for chemicals.
- Cereals for feed and DDGS (dried distillers grains and solids) could also be biorefined to yield feed with optimal amino acid levels and proteins for chemical production.

The above shows that biorefinery of grass and high protein feeds could yield at least 3.75 + 6.25 = 10 million tons of protein for chemicals. Bone and meat meal could also yield 1 million tons of protein for chemicals.

More protein for chemicals could be sourced from the by-products of the expanding biofuel production. This includes DDGS and rape meal.

Another option is specific protein crop production. Still, a good comparison to the chemical route would have to made to see if this is economically energetically and especially environmentally beneficial. Crops that could be used include alfalfa (luzerne), peas and lupins.

Production of proteins by algae should also be an option especially of the N source is manure from intensive animal production.

Conclusions

Protein containing biomasses have not only been used for food and feed, but also for non-food applications. In technical applications, the use of proteins, however, is limited and restricted to high-added-value markets like coatings, labelling adhesives, surfactants and in cosmetic products.

The last years, the amount of biomass streams that contain proteins is increasing. For example, the increased production of oil from oilseeds for biodiesel production goes together with an increased production of press cakes. These protein containing press cakes are now mainly being used as animal feed. The prices of soy, rape and sunflower cake are about 150-300 €/ton. The prices of soy proteins concentrates and isolates are, however, much higher; between 2000 and 3000 €/ton. Based on this, biorefinery approaches by which proteins are being isolated from all kind of biomass streams seems to be an interesting option to enhance the economical value of these biomass streams. However, it has to be taken into account that the economical value of proteins is depending on their functional properties. To use protein as a bulk polymer in technical applications or e.g. as emulsifier in food systems, it is important that proteins have for a large part maintained their native properties. In cases that proteins are denatured or cross-linked in industrial processes, probably the most relevant use for these proteins, is as a starting material for the production of chemicals. In that case, the back bone is hydrolysed into amino acids which, after isolation, can be converted into chemicals. For example, the wheat gluten proteins that are present in DDGS (bio ethanol process) have been severely modified by Maillard reactions and have lost all their functional properties.

Another strategy could be to modify industrial processes, taking into account different biorefinery aspects, in such a way that proteins are less destroyed by the process. For example, changes in process temperature or isolation of proteins prior to the “main” process could be an option.

In addition, protein resources like aquatic biomass and grass will be subject of protein isolation studies. At this moment micro algae projects are running focussing on oil production. Half of the biomass, however, is protein and therefore the isolation of these proteins and determination of their chemical and physical properties an interesting subject.

As a follow up from this study, one specific promising case could be further studied by which market demands of companies can be coupled to available biomass.

References

Barbarino, E., S. Lourenco, et al. (`2005). "An evaluation of methods for extraction and quantification of protein from marine macro- and microalgae." J. Applied Phycology **17**: 447-460.

Becker, E. (2007). "Micro-algae as a source of protein." Biotechnology advances **25**: 207-210.

Bos, H., W. Elbersen, et al. (2010). "Market demand for non-food crops." Report 4FCrops.

Dominguez, H., M. J. Nunez, et al. (1994). "Enzymatic Pretreatment to Enhance Oil Extraction from Fruits and Oilseeds - a Review." Food Chemistry **49**(3): 271-286.

Duke, J. "Handbook of energy Crops." http://www.hort.purdue.edu/newcrop/indices/index_ab.html.

Kamm, B., P. Gruber, et al. (2006). "Biorefineries-Industrial Processes and products. Status quo and future directions." Wiley-VCH Verlag GmbH & Co. KGaA.

Ladepeche (2010). www.depeche.fr.

Lestari, D., W. Mulder, et al. "Improving Jatropha Curcas seed protein recovery by using counter current multistage extraction." article in press.

Leuchtenberger, W. (1996). Amino acids - technical production and use. Weinheim, VCH.

Leuchtenberger, W., K. Huthmacher, et al. (2005). "Biotechnological production of amino acids and derivatives: current status and prospects." Appl Macrobiol Biotechnol **69**: 1-8.

Massoura, J., J. Vereijken, et al. (1998). "Proteins from Crambe abyssinica Oilseed.
I. Isolation Procedure." Journal of the American Oil Chemists' Society **75**(3): 323.

Nasi, M. (1983). "Extraction of leaf protein from green crops. Chemical composition and nutritive value of products of fractionation." Journal of the scientific agricultural society of Finland **55**: 143-154.

Nordisk, N. (1997). "WO98/18343."

Ordonez, C., C. Benitez, et al. (2008). "Amino acid production from a sunflower wholemeal protein concentrate." Bioresource Technology **99**: 4749-4754.

Perez, S. G. (2003). "Physico-chemical and functional properties of sunflower proteins." Thesis.

Prolea (2008). www.prolea.com.

Reith, J., E. Deurwaarder, et al. "BIO-OFFSHORE
Grootschalige teelt van zeewieren in combinatie met
offshore windparken in de Noordzee." ECN-C--05-008.

Rosenthal, A., D. L. Pyle, et al. (1996). "Aqueous and enzymatic processes for edible oil extraction." Enzyme and Microbial Technology **19**(6): 402-420.

Schrooyen, P. (1999). "Feather keratins: Modification and film formation." Thesis.

Scott, E., F. Peter, et al. (2007). "Biomass in the manufacture of industrial products - the use of proteins and amino acids." Applied Microbiology and Biotechnology **75**(4): 751-762.

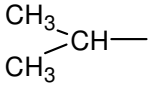
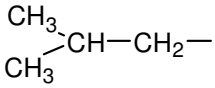
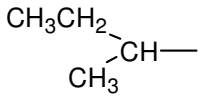
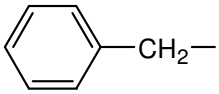
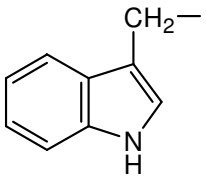
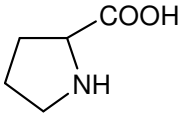
Werpy, T. and P. G. (2004). "Top value added chemicals from biomass. PNNL, NREL, EERE (report 8674)."

Zhang, S. B. (2007). "Downstream processes for aqueous enzymatic extraction of rapeseed oil and protein hydrolysates." J. Am. Oil Chem. Soc. **84**: 693-700.

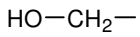
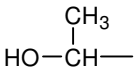
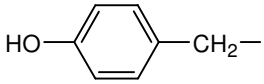
Zhang, S. B., Z. Wang, et al. (2007). "Optimization of the aqueous enzymatic extraction of rapeseed oil and protein hydrolysates." Journal of the American Oil Chemists Society **84**(1): 97-105.

Appendix: Protein structure

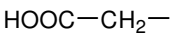
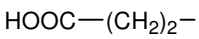
The twenty alpha amino acids that occur in proteins

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

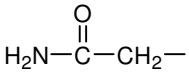
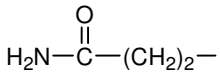
Side chains with a hydroxyl group

Serine	Ser	S		5.68
Threonine	Thr	T		5.64
Tyrosine	Tyr	Y		5.66

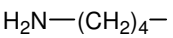
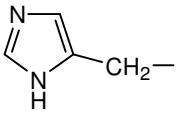
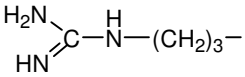
Side chains with a carboxyl group

Aspartic acid	Asp	D		2.77
Glutamic acid	Glu	E		3.22

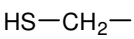
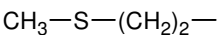
Side chains with an amide group

Asparagine	Asn	N		5.41
Glutamine	Gln	Q		5.65

Side chains with a basic group

Lysine	Lys	K		9.74
Histidine	His	H		7.59
Arginine	Arg	R		10.76

Side chains with a sulfur containing group

Cysteine	Cys	C		5.07
Methionine	Met	M		5.74

The structure of proteins

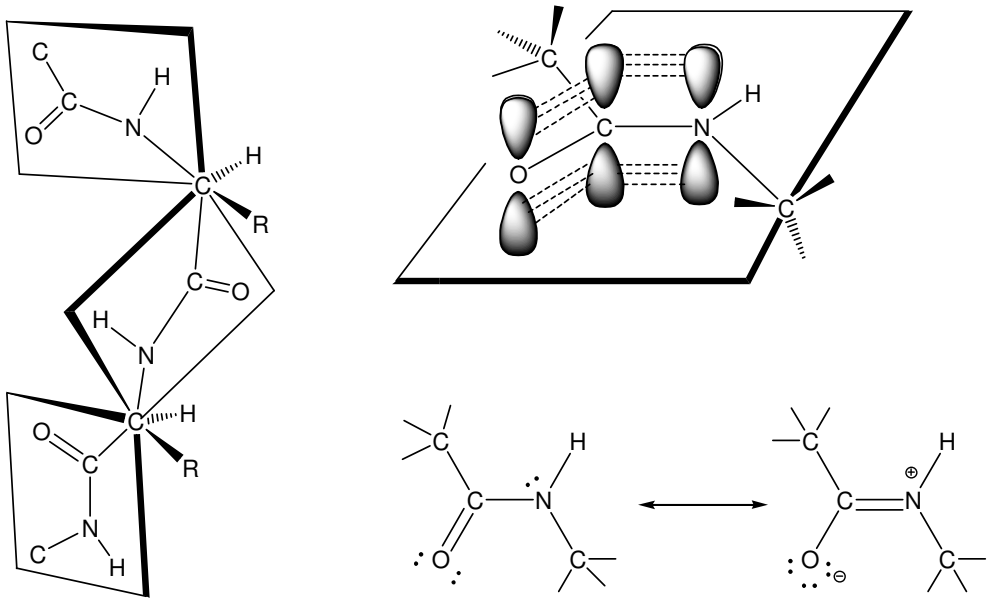
Primary structure:
sequence of amino acids in the chain

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

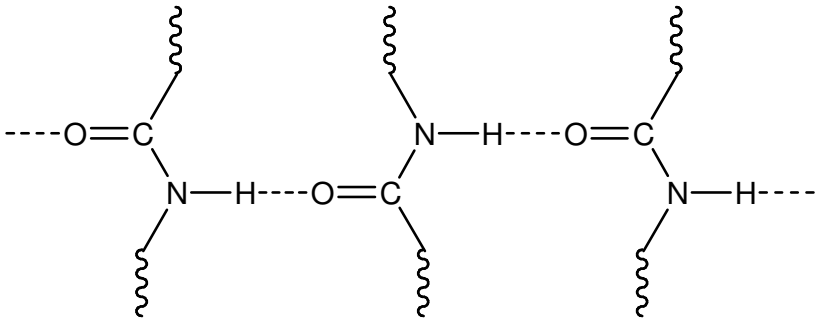
Tertiary structure:
the fold of the individual secondary structures in 3D, mainly maintained by interactions between the amino acid side chains

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

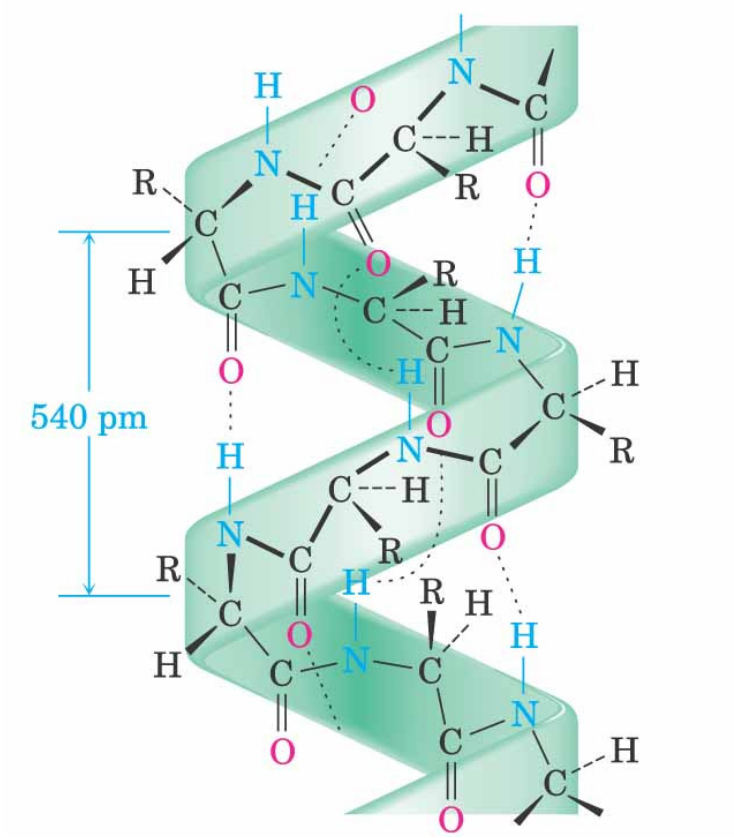
Secondary structure



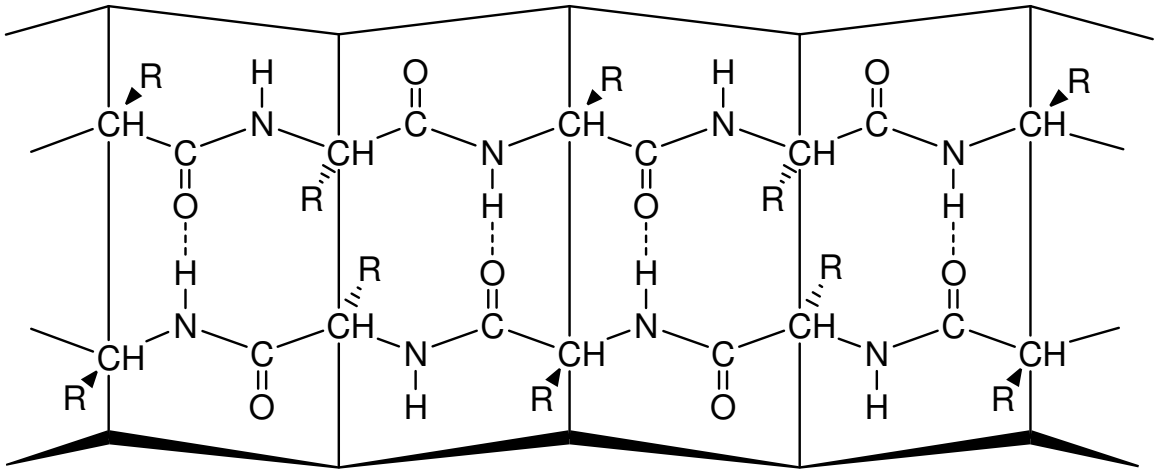
Trans orientation of the chain around the peptide bond



Example of secondary structure

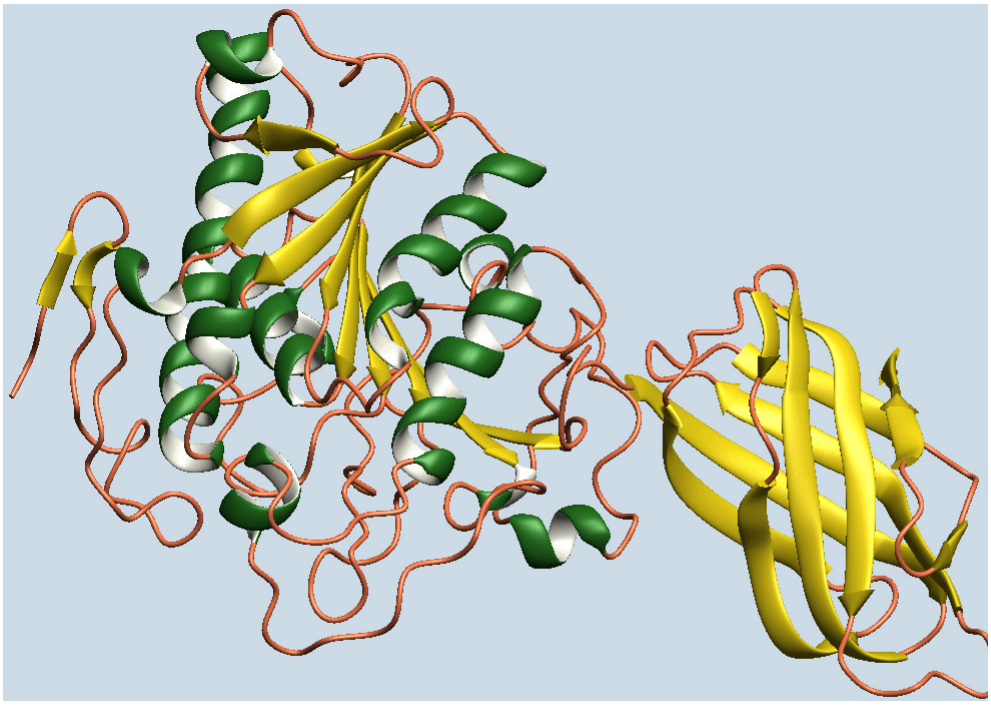


α helix



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

Colofon

Dit rapport is een uitgave in opdracht van het Platform Groene Grondstoffen van EnergieTransitie.

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