HANDBOOK OF HYDROCOLLOIDS. Second edition

Edited by G.O. Phillips and P.A. Williams

Woodhead Publishing in Food Science, Technology and Nutrition

Woodhead Publishing Limited
ISBN 978-1-84569-414-2

Oxford Cambridge New Delhi

VEGETABLE PROTEIN ISOLATES

S. González-Pérez and J. B. Arellano
Instituto de Recursos Naturales y Agrobiología de Salamanca (IRNASA-CSIC), Apdo. 257, 37071 Salamanca, Spain.

Abstract: This chapter discusses the importance of vegetable proteins as functional ingredients in food formulations. It provides an overview of the main chemical components, world production, history and food applications of the main vegetable protein sources (legumes, cereals, oilseeds, roots and green leaves), with special emphasis on soybean, pea, and wheat. The chapter includes a description of the composition and structure of the main protein fractions, and a general approach to protein extraction, purification and processing technology to prepare protein meals, concentrates and isolates. In addition, the technologically important functional properties of vegetable protein preparations are described. Finally, nutritional and health effects, as well as the regulatory status of vegetable protein products are examined.

Key words: vegetable protein, food applications, meal and isolate, functional properties, protein structure and composition.

15.1 Introduction

The worldwide demand for proteins is increasing and, as a consequence, there is a need for new sources of food proteins. Animal proteins are expensive in terms of market price, land requirement and environmental impact. In addition, consumers' confidence in animal proteins has decreased due to food safety problems related to diseases such as bovine spongiform encephalopathy and the use of animal hormones. The conversion of vegetable into animal protein entails considerable losses in protein, water and energy. Under industrial conditions, the energy consumption per kilogram of animal protein is 8–10 times higher than for vegetable protein. Furthermore, the rising prices for raw materials and energy press market to the production of low-cost high quality protein foods.

Vegetable proteins are economic and versatile alternatives to animal proteins as functional ingredients in food formulations. Nevertheless, an effective replacement of animal proteins requires technological innovations. To achieve these innovations in an effective and efficient way, insight into the relation between protein structure and their functional properties is of prime importance. The main sources of vegetable proteins currently under scientific and technological study and/or already in the market are the following:

- Legume grains as peas (*Pisum sativum*), soybeans (*Glycine max*), lupins (*Lupinus* spp), chickpeas (*Cicer arietinum*) or peanuts (*Arachis hypogaea*).
- Cereals as wheat (*Triticum* spp), maize (*Zea mays*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), rye (*Secale cereale*), oat (*Avena sativa*) or sorghum (*Sorghum* spp).
- Oilseeds as sunflower (*Helianthus annuus*), rapeseed (*Brassica napus*), sesame (*Sesamum indicum*), cottonseed (*Gossypium* spp), or safflower (*Carthamus tinctorius*).
- Root vegetables as potato (*Solanum tuberosum*), cassava (*Manihot esculenta*) or sweetpotato (*Ipomoea batatas*).
- Leaves from alfalfa (*Medicago sativa*), cassava, amaranth (*Amaranthus* spp) or aquatic plants.
- Fruits as grape seed (*Vitis vinifera*), tomato seed (*Solanum lycopersicum*) or papaya kernel (*Carica papaya*).

15.1.1 Legumes

Legumes have played a vital part in many ancient civilizations. The use of legumes as a basic dietary staple can be traced back more than 20,000 years in some eastern cultures. Legumes are crop plants from the family *Fabaceae* (or *Leguminosae*). The key characteristic of legume plants is the ability, of nearly all its members to fix atmospheric nitrogen to produce their own protein compounds thanks to the symbiotic association with nitrogen-fixing bacteria (i.e. rhizobia) found in the root nodules.

Farmed legumes can be basically classified in two groups: forage legumes and grain legumes. Forage legumes are grown for grazing or hay, and sometimes for industrial purposes. Grain legumes are cultivated for their grains (Fig. 15.1), which are harvested at maturity and marketed as dry product.

Legume foliage and grains have comparatively higher protein content than non-legume material, making them attractive crops in agriculture.

There are about 18,000 legume species. More than 40 species and countless varieties of grain legumes are cultivated throughout the world. Pea is the main cultivated protein crop in Europe. Other major grain legumes grown in Europe are faba beans (*Vicia faba*), lentils (*Lens culinaris*), soybeans, lupins and chickpeas. However, three-quarters of the world production of grain legumes is soybeans, grown primarily in the United States, Brazil, Argentina, Paraguay and Uruguay. The world production of soybeans has increased by 215% over the last 30 years compared with just 50% for other grain legumes.

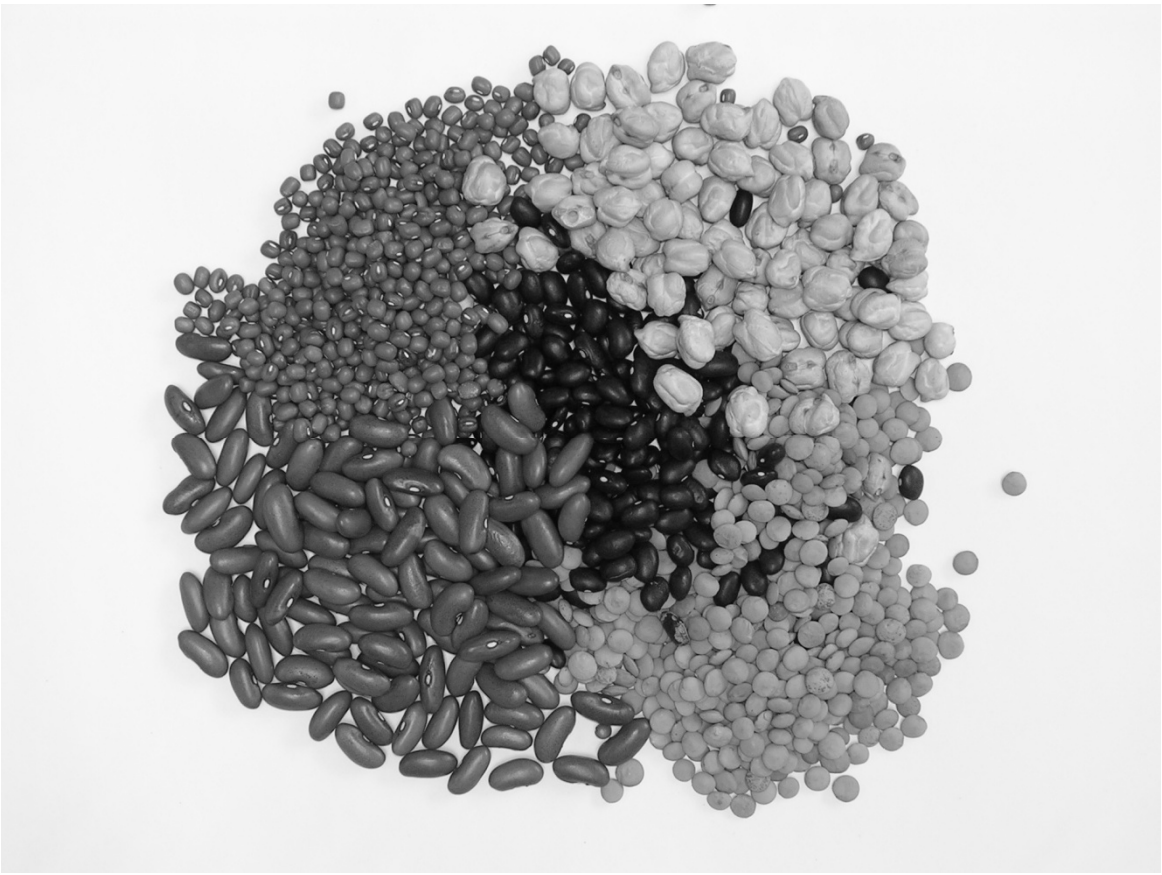

Fig. 15.1 Some popular grain legumes.

Soybean

Soybean is one of humanity's main food crops. It is widely accepted that soybeans originated in China around 5,000 years ago. Cultivation of soybeans, confined mainly to China, gradually extended to other continents. It was first introduced to Europe at the beginning of the eighteenth century. Soybeans were brought to America in the late eighteenth century, but a large-scale official introduction did not take place until the early 1900s. In 1920, combines were first used to harvest soybeans, and in 1922, the first soybean processing plant opened. By 1992, the United States accounted for half of the total world soybean production.

Pea

Native to Southwest Asia, it was among the first crops cultivated by man. Wild field peas of related species can still be found in Afghanistan, Iran, and Ethiopia. Peas were an important staple crop throughout Medieval Europe. It was introduced into America soon after Columbus's expedition. It is a cool season crop and is widely grown in the cooler temperate zones of the world, such as Northern Europe.

15.1.2 Cereals

The first recorded cereal farming communities were found 9,000 BC in the region between the Tigris and Euphrates rivers. Wheat, barley and rice were grown around 2,000 BC in northern India. Cereal crops or grains are edible seeds of plants belonging to the grass family (*Gramineae*). Cereals are monocotyledonous angiosperms and are cultivated worldwide, from semi-arid conditions to very hot and cold regions. They are a major source of dietary protein for humans, on average representing about 50% of the protein consumed worldwide. The mean annual production of cereals for 2001–2005 surpassed 2,100 million tonnes. The main cereals crops are wheat, rice, maize, barley and sorghum. Wheat, rice and maize account for 85% of the cereal production.

Wheat

Wheat has been used as a food since early human history and it is among the oldest and most extensively grown of all crops. Historical evidence points to the cultivation of wheat in the Nile valley around 5,000 BC, the Indus and Euphrates valleys by 4,000 BC, China by 2,500 BC and England by 2,000 BC. Wheat cultivation expanded from Mediterranean centres of leading agriculture, towards Asia and Europe. Wheat is the most important and widely cultivated cereal crop in the world; 31% of the cereal production is wheat. It is grown in very different climates, from the arctic cold of Russia to the extreme heat of India. Different classes of commercial wheat cultivars are distinguished according to technologically relevant properties such as kernel hardness, bran colour and protein content. Typical distinctions are hard or soft, strong or weak.

15.1.3 Oilseeds

Oilseeds are seeds grown primarily for the production of edible oils. In the broader sense, peanuts and soybeans can be considered oilseeds. The history of oilseeds is closely tied with that of human civilization. Rapeseed and sesame were mentioned in the Indian Sanskrit writings of 2,000 BC, and sunflower was reported to be present in Arizona and New Mexico 3,000 years BC. Vegetable oil is obtained by pressing the seeds and then extracting the oil. The cake or meal obtained in the extraction is a rich source of proteins: sunflower and peanut meals contain 40–50% and 50–60% proteins, respectively. Other oilseed meals contain 35–45% proteins. Soybean, rapeseed, cottonseed, sunflower and peanut represent a 69, 12, 7, 5 and 3% of world protein meal production, respectively. World oilseed production reached 380 million metric tonnes in 2004/2005.

15.1.4 Root vegetables

Root vegetable is a very general definition for a wide cross-section of subterranean storage organs. Root vegetables include both true roots such as tuberous roots and taproots. Roots and tubers were already important in the diet during the early evolution of mankind. With the advent of agriculture, cultivated root and tuber crops became increasingly critical sources of food, with potato, cassava and sweetpotato representing the 3rd, 6th and 7th most important sources of food for humans worldwide. Root vegetables are rich in carbohydrates but poor in protein content.

Potato

Potato belongs to the family of *Solanaceae*. It has been cultivated for thousands of years in South America and it was introduced into Europe in the second half of the sixteenth century. Nowadays, potato is the fourth most major crop for human consumption after wheat, rice and maize. The main industrial outlet of potato is starch production. The by-products that remain after starch manufacture are the fibres and the so-called potato juice, wherein proteins are found. Indeed, potato ranks second to soybean in the amount of protein produced per hectare. However, it is chiefly use in animal feed due to its poor solubility after the currently applied industrial process. For this reason the contribution of proteins to the total value of the industrially used potato is limited.

15.1.5 Green leaves and fruits

Protein synthesis is one of the chief activities of the green part of the plant. Some forage crops produce leaf protein in large quantities, and therefore, leaf protein concentrate (LPC) is expected to be a valuable protein supplement for the human diet. LPC was first suggested as a human food in the 1960s, but it has not achieved much success, despite early promise. LPC has been rejected for human consumption due to

its bitter, grassy flavour, and dark green colour. Most research has been on alfalfa, amaranth, aquatic plants (e.g. duckweed), and by-product leaves (e.g. faba bean, pea).

The continuous development of new food proteins from secondary and new sources has also found its way into using fruit by-products such as date (*Phoenix dactylifera*) seeds, grape seeds, tomato seeds, papaya, mango (*Mangifera* spp) and apricot (*Prunus armeniaca*) kernels. Tomato seeds are a valuable by-product from the tomato paste manufacturing industry, with a protein content of about 20–30%. Furthermore, the nutritional and functional characteristics of tomato seeds have been found comparable to commonly used vegetable proteins.

15.2 Chemical composition of vegetable proteins

Plants contain a wide range of chemical compounds and display considerable variation in composition. Apart from the obvious interspecies differences, the rate and extent of chemical change depend on the growing conditions, the physiological role of the plant part, the genotype, and the post-harvest environment.

15.2.1 Legumes

In general, legumes are sources of complex carbohydrates, protein and dietary fibre, having significant amounts of vitamins and minerals. Legume seeds are characterized by relatively high protein content, from 17 to 40%.

Soybean

Soybean comprises about 8% seed coat or hull, 90% cotyledons and 2% germ. The proximate composition of soybeans is about 40% proteins, 35% carbohydrates, and 20% lipids. A variation ranging from 13 to 23% in lipids and 32 to 50% in protein has been described among different cultivars.

Pea

The protein content of peas ranges from 20 to 25%. Peas also contain about 33–50% starch, but are low in fat. Pea protein is a good source of essential amino acids, having high lysine content, but is limiting in tryptophan and in the sulphur-containing amino acids.

15.2.2 Cereals

Cereal grains consist of 12–14% water, 55–75% carbohydrates, 7–13% proteins and 1–6% lipids. Oats and maize, however, contain relatively large amounts of lipids. Table 15.1 provides the average proximate composition of some cereal grains. However, the data given should be used only as a guide because of the large differences reported in composition. For instance, the protein content of wheat may range between 8 and 18%.

Wheat

The chemical components of cereals are not homogeneously distributed in the kernel. Hulls and bran are high in cellulose and pentosans. The aleurone layer of wheat contains 25 times more minerals than the endosperm, and lipids are more abundant in the aleurone and germ. The endosperm contains mainly starch and a lesser amount of protein than the germ and the bran.

Table 15.1 Approximate composition (%) of various cereal grains

Grain	Protein	Lipids	Fibre	Carbohydrates
Wheat	11	2	2	70
Rice	7	2	1	64
Maize	10	**5**	2	63
Barley	11	3	3	56
Sorghum	8	4	4	63
Rye	9	2	2	72
Oats	9	6	2	63

15.2.3 Oilseeds

The proportion of hull and kernel in oilseeds varies considerably and affect the final chemical composition. Oil and proteins are the main components of oilseeds. Approximately 16–40% of the weight of oilseeds is protein and 18– 45% is lipids. These values are strongly affected by the oilseed variety.

15.2.4 Potatoes, green leaves and fruits

Potatoes contain 75% water and 25% dry matter. Potatoes are best known for their carbohydrate content, being starch, the major form of carbohydrate. The protein content ranges from 0.7–4.6% and lipid content from 0.02–0.96%.

The composition of vegetables can vary significantly depending on the cultivar and origin. Dry matter comprises about 10–20% in most vegetables, of which 3–20% are carbohydrates, 1–5% proteins, 1% fibre, 0.1–0.3% lipids and close to 1% minerals.

Fruit composition is strongly affected by the variety and ripeness. In general terms the dry matter content varies between 10 and 20%, sugars, polysaccharides, and organic acids being the main constituents. Proteins and lipids are present in much lesser amounts. In nuts, however, the moisture content is below 10%, nitrogen compounds are about 20%, and lipids can be as high as 50%.

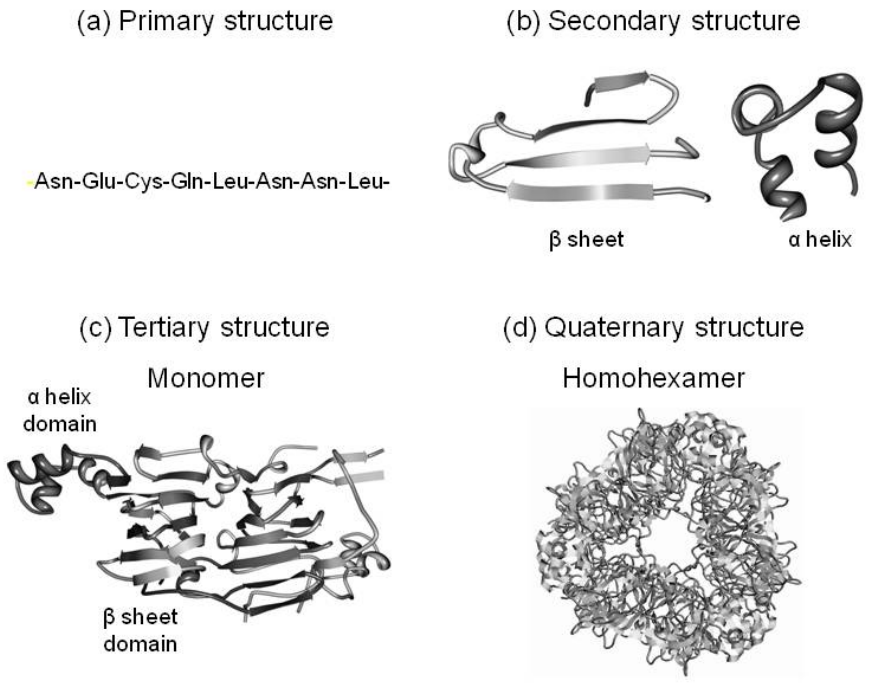

Fig. 15.2 Protein structure of soybean 11S globulin: homohexamer of the glycinin A3B4 subunit (PDB: 1OD5).

15.3 Protein composition and structure

Proteins are complex macromolecules. The linear sequence of amino acids in a protein is known as the primary structure. The primary structure provides valuable information (i.e. molecular weight, ratio hydrophobic/hydrophilic residues, etc.), but far insufficient insight to understand functionality of food products. Besides the primary sequence, conformational folding determines, in a very complex way, the secondary, tertiary, and quaternary structure of proteins, providing information about the actual extent of exposure of hydrophobic regions, apparent net charge, size, shape, etc. Figure 15.2 shows the primary, secondary, tertiary and quaternary structure of soybean glycinin.

Protein content and amino acid composition vary considerable between families, genera, species and cultivars. The parameters are also dependent on the growth stage of the plant, as well as on the part (tissue) under analysis. Most proteins described in this chapter occur in seeds/grains. The usage value of grains from the main crops (cereals, oilseeds and legumes) depends on their composition and on the organization of components (from molecular to macroscopic scale) in the storage organs. Seed proteins can be roughly classified into three groups:

1. Storage proteins.
2. Biological active proteins, such as lectins, enzymes and enzyme inhibitors.
3. Structural proteins, such as ribosomal, chromosomal and membrane proteins.

The storage proteins are the most abundant in seeds and are, therefore, the key components of grain quality. They comprise several protein types with particular structures and (technological/biological) functionalities. There is no clear definition for storage protein, and generally a protein present in an amount of 5% or more of the total protein fraction is considered a storage protein. Storage proteins have also been defined as a group that comprises proteins generated mainly during seed development and stored in the seed to serve as a nitrogen source during germination. Other properties that are generally attributed to storage proteins are the absence of enzymatic activity, the occurrence in a membrane surrounded vesicle (protein bodies or aleurone grain), and the predominance of a multimeric structure. Finally, a further feature of storage proteins is the polymorphism of their polypeptides, both within single genotypes and among genotypes of the same species. This polymorphism is due to the presence of multigene families and to the occurrence of post-translational proteolitic cleavage and glycosylation.

15.3.1 Classification of storage proteins

Seed proteins were empirically classified by Osborne (1924) based on the sequential extraction of crushed and defatted seeds by aqueous and non-aqueous solvents. According to this classification, albumins are soluble in water. Globulins are soluble in diluted salt solutions. Prolamins are alcohol-soluble and glutelins are the most difficult to solubilise; they are extractable by weak alkaline, weak acidic and dilute detergent solutions. However, it should be emphasised that the classification according to this scheme depends on the conditions during meal preparation, seed pre-treatment, and on the way the fractionation is performed (e.g. time of extraction, liquid to seed ratio, pH, etc.).

Albumins and globulins are the main storage proteins of dicotyledonous plants (e.g. legumes, oilseeds), whereas prolamins and glutelins are major proteins in monocotyledonous plants (e.g. cereals). As expected of a nitrogen source, storage proteins are rich in asparagine (and aspartate), glutamine (and glutamate) and arginine.

Albumins

This group of proteins is characterized by a low molecular weight, sedimentation coefficients of approximately 2S (S stands for the Svedberg unit), high solubility in water and to be compact globular proteins. In addition to high nitrogen content, they are rich in cysteines. Albumins belong to a widely distributed family of seed proteins and their molecular weight ranges from about 10 to 18 kDa. They have been reported to account for 20 to 60% of the total proteins in seed of dicotyledonous plants. Albumins consist of two polypeptide chains (synthesized from a single precursor protein that is proteolytically cleaved) linked by disulfide bonds, except for lupin that lacks the interchain disulfide bonds and sunflower where the 2S protein remains uncleaved.

Globulins

Globulins have been extensively characterized, particularly in nutritionally important legumes and oilseeds. They represent the major storage protein of legumes and oilseeds. The sedimentation coefficients of globulins range from 7S to 12S. The major globulin fractions sediment at approximately 7S (vicilin-like globulins) and 10–12S (legumin-like globulins).

Vicilin-like proteins are regarded as the second major group of seed globulins. The molecular weights are in the 150–190 kDa range and are typically trimeric proteins consisting of subunits of various molecular weights. They lack cysteine residues and consequently cannot form disulfide bonds. Moreover, 7S globulins are generally glycosylated in contrast to 10–12S globulins (with the exception of 12S from lupin and amaranth). As it occurs for the other storage proteins, the vicilin-like proteins are usually

assigned descriptive names based on the source as vicilin (pea), β-conglycinin (soybean) or acalin A (cottonseed).

The legumin-like globulins are the major fraction in legumes and oilseeds. Legumin-like globulins are generally designated by names according to their plant origin as glycinin (soybean), arachin (peanut) or helianthinin (sunflower). A high similarity has been found among the legumin-like globulins, with respect to molecular weight (300–370 kDa), size, shape, charge, solubility and other characteristics. They are hexameric proteins consisting of six acidic (α-) polypeptides and six basic (β-) polypeptides, generally disulphide linked in pairs (α,β) to form monomers of about 60 kDa. The most accepted model for the quaternary structure of legumin-like globulins is an arrangement of six spherical subunits into a trigonal antiprism with a maximum dimension of 11 nm. This model was determined by electron microscopy and small angle X-ray scattering. In spite of the large diversity in polypeptides and amino acid sequences of the legumin-like globulins from different species, various seed proteins share regions that are conserved in sequence and/or structure. Similarities have been found in quaternary and secondary structure; however, substantial differences have been observed in the tertiary structure of seed globulins.

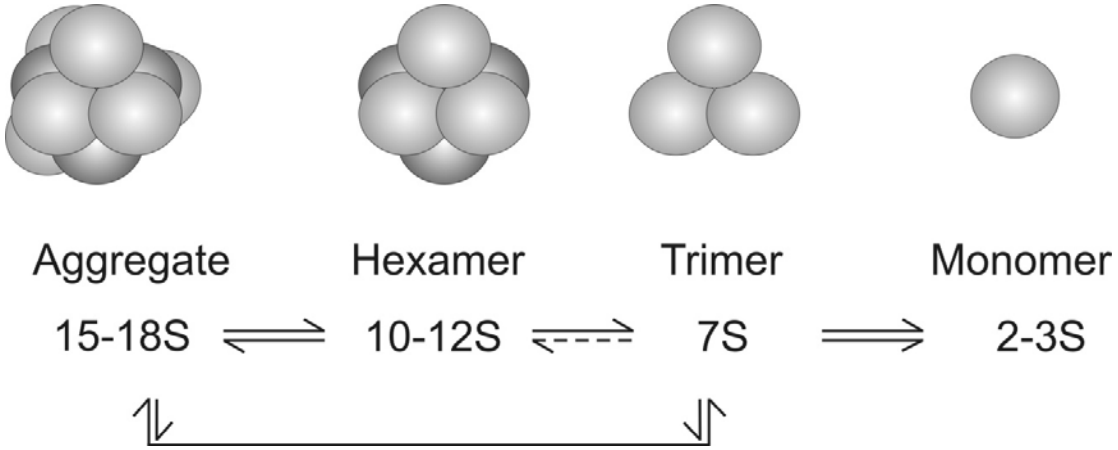

Fig. 15.3 Schematic model for the quaternary structure association-dissociation phenomena of legumin- and vicilin-like globulins.

Although the 7S and 10–12S globulins show no obvious sequence similarities, they do have similar properties, including the ability to form both trimeric and hexameric structures. Another important characteristic shared by globulins is that their quaternary structures are modulated by both ionic strength and pH. They undergo reversible and irreversible association-dissociation phenomena, which have complicated their isolation and characterization (Fig. 15.3). For instance, the majority of 7S globulins from mono- and dicotyledonous seeds associate to larger molecular weight species (9–12S) at low ionic strength (I = 0.1 M). The relatively easy association-dissociation indicates that the subunit interactions are non-covalent in nature (i.e. hydrogen bonds, electrostatic, hydrophobic and van der Waals).

Prolamins and glutelins

Prolamins and glutelins are both hydrophobic and specifically accumulate in small vacuoles or protein bodies. In addition to poor water solubility, they are characterized by high contents of proline and glutamine. Prolamins are the major storage protein in cereals except for rice and oats, where glutelins and globulins are the major proteins. Prolamins are assigned trivial names according to the species of origin, such as gliadin (wheat), hordein (barley), zein (maize), secalin (rye) or avenin (oat). The cereal prolamins are present as monomers or small aggregates, while glutelins form large disulphide-bonded aggregates.

15.3.2 Soybean proteins

Glycinin (11S globulin) and β-conglycinin (7S globulin) are the most important soybean proteins. The 2S fraction is found in a lesser amount and consists of Bowman-Birk and Kunitz trypsin inhibitors, cytochrome *c*, and α-conglycinin. As other legumin-like globulins, glycinin consists of one basic and one acidic polypeptide, which are linked by a single disulphide bond, except for the acidic polypeptide A4. The molecular masses of the basic polypeptides are around 20 kDa, while those of the acidic polypeptides are around 38 kDa. At ambient temperatures and pH 7.6 (I = 0.5 M), glycinin forms hexamers (11S) with

molecular masses around 300–360 kDa, while at pH 3.8 glycinin is present as trimers (7S) with a molecular mass of about 180 kDa. Lowering the ionic strength at neutral pH from 0.5 to below 0.1M induces dissociation of 11S glycinin into 7S glycinin.

β-Conglycinin is a trimeric glycoprotein (7S) consisting of three types of subunits, α' (57–72 kDa), α (57–68 kDa) and β (45–52 kDa) in different combinations. As for other 7S globulins, the subunits are associated via hydrophobic and hydrogen bonded interactions without the contribution of disulphide bonds. At pH 5 and higher, β-conglycinin is found as a trimer (I = 0.5 M), whereas it predominantly exists as a hexamer at ionic strength lower than 0.1 M. At pH values 2–5, β-conglycinin reversibly dissociates into a 2–3S and 5–6S fraction at ionic strength lower than 0.1 M.

15.3.3 Pea proteins

Dry pea seeds contain about 25% proteins, of which 70–80% are globulins: legumin, vicilin and convicilin. Legumin comprises six subunits with a molar mass of about 60 kDa. Each subunit is proteolitically cleaved into disulfide-linked basic and acidic polypeptides. These polypeptides display charge and size heterogeneity, reflecting the production of legumin from a small gene family. Four/five acidic and five/six basic polypeptides have been identified. The sizes of these polypeptides range from 38 to 40 kDa for the acidic ones and from 19 to 22 kDa for the basic polypeptides.

Vicilin, the 7S pea protein, is generally found as a trimer, each subunit having a molar mass of about 50 kDa. Vicilin can be cleaved at one or two sites (called the α-β and β-γ processing sites) as specified by the coding sequence of the vicilin genes. Cleavage at the α-β site generates fragments of 19 and 30 kDa, whereas that at the β- γ site generates fragments of 12.5 or 16 and 33 kDa. Cleavage at both sites generates fragments of 12.5, 13.5 and 16 or 19 kDa. The small fragments of vicilin contribute to the extensive polypeptide heterogeneity of vicilin. Other factors are the large number of vicilin genes in the pea genome, differential glycosylation, and surface charge heterogeneity (around the potential site of cleavage).

Pea convicilin is a 7–8S trimeric protein containing polypeptides with molecular mass of about 70 kDa. Convicilin has an extensive homology with vicilin along the core of its protein, but holds an additional highly charged, hydrophilic sequence of 120–166 residues close to the polypeptide N-terminus. Convicilin also contains one cysteine residue contrary to the majority of 7S globulins.

15.3.4 Wheat proteins

Some 80% of wheat proteins is gluten. Albumins and globulins are minor wheat proteins (20%). Gluten largely consists of two groups of proteins, the sulphur-rich glutenins, able to form a polymeric network by chemical crosslinking and the sulphur-poor gliadins, largely present as monomers that interact with the glutenin in the network. Glutenins affect the elastic properties of dough while gliadins affect the viscous properties of dough. The glutenin fraction consists of high molecular weight glutenin subunits with molecular masses from 80 to145 kDa and low molecular weight glutenin subunits, having molecular masses from 31 to 48 kDa. There are similarities between both subunits in terms of solubility, sequence and conformation.

15.3.5 Potato and green leaf proteins

Soluble potato tuber proteins have been classified into three groups: (i) patatin, the major protein, (ii) protease inhibitors, and (iii) a third group containing all other proteins. Patatin is a 45 kDa glycoprotein that represents 40–60% of all buffer-extractable tuber proteins. It is considered to be a storage protein because of its high accumulation in the tuber.

Rubisco (Ribulose-1,5-bisphosphate carboxylase/oxygenase) is the most abundant protein in leaves. It accounts up to 30–70% of soluble leaf proteins. It is an enzyme from the Calvin-Benson-Bassham cycle that catalyses the first major step of the photosynthetic CO2 assimilation. Rubisco is a 550 kDa globular protein with a general structure L_8S_8 consisting of two types of protein subunit, the large chain (L, ~55 kDa) and the small chain (S, ~12.5 kDa).

15.4 Manufacture

In order to design a process to prepare a protein isolate, some considerations should be taken into account. It is important to know the amount of protein required, the degree of final purity, whether loss of activity is acceptable, and the time and cost of the purification. Other factors to consider are the source material,

i.e. species, seasonal variation and development stage of the crop, structure of the raw material (seeds, leaves, hulls, etc.), and previous processing.

The first step in protein isolation requires disruption of the tissues in which the proteins are compartmentalized. The next step is the preparation of an extract containing the protein in a soluble form. The efficiency of the method depends largely on the differential solubility of the various components (protein, carbohydrates, pigments, fibres, etc.) as a function of temperature, pH and ionic strength. Extremes pH values may cause protein denaturation, functional and nutritional adverse reactions. For instance, extraction at extreme basic pH values may cause racemization of amino acids, reduced protein digestibility, and losses of essential amino acids such as cysteine and lysine. For most plant sources, pH values between 3 and 9 should not be harmful for proteins. Proteins from oilseeds and legumes are generally soluble in aqueous media, whereas cereal proteins are much more insoluble. For the latter, the use of surfactants or organic solvents such as ethanol is usually required. Studies of protein solubility usually precede development of large-scale processes, though published information on protein solubility is not always applicable since it refers to high solvent/protein ratios. In large-scale processing the volumes of solvent have a considerable effect on equipment costing and handling.

Once the protein has been extracted, clarification is generally used to separate the protein from the unwanted material. Centrifugation, filtration and membrane processes are the most common techniques for clarification. Nowadays, tangential or cross-flow microfiltration is receiving increased attention, principally for large-scale applications. After the clarification step, the protein is in solution, but its concentration can be very dilute. The next step is to concentrate the clarified protein solution quickly, while keeping it in a stable state, generally by ultrafiltration or precipitation. Protein products concentrated by ultrafiltration are generally of better functional quality than those obtained by precipitation. Precipitation is carried out by isoelectric precipitation, organic solvents, addition of salts (salting-out method), reduction of ionic strength (salting-in method), exclusion polymers or heating.

Isoelectric precipitation is the precipitation method most commonly used. The greatest disadvantage of isoelectric precipitation is that it can lead to extensive protein aggregation and modification of protein solubility, mostly caused by non-covalent interactions. The latter is particularly problematic in large-scale operations since the use of concentrated acids or alkalis, to reduce handling volumes, may result in denaturation by local, transitory extreme pH values. For this reason isoelectric point precipitation is sometimes used to precipitate contaminant proteins, rather than the target protein.

Organic solvents may cause protein precipitation. This method is commonly used by the blood fractionation industry (Cohn process). For instance, for vegetable protein isolation, the use of water/alcohol mixtures is a common concentration step, as soluble carbohydrates, pigments, flavours and saponins are removed. A drawback of the treatment with organic solvents is that it often leads to protein denaturation, so such solvents are generally avoided for recovery of intact protein.

Salting-out methods for protein recovery are scarcely employed commercially because by-product recovery costs are too high. Isolates from salting-in concentration have comparable yield to those produced from isoelectric precipitation, with the advantage that protein denaturation is avoided. Extracted proteins can be precipitated as well by specific cations, e.g. calcium in tofu manufacture, although it renders poor functional isolates because of the difficult resolubilization of the gel formed.

Table 15.2 Approximate composition (%) of the soybean defatted meal, concentrate and isolate

	Defatted meal	Concentrate	Isolate
Protein	55	70	>90
Lipids	1<	1<	1<
Carbohydrates	32	20	3
Fibre	3	4	1<

The exclusion of proteins from high polymer media consists in the addition of neutral polymers (dextroses, PEG, etc.) to protein dispersions resulting in separation of a protein-rich phase. For instance, the addition of PEG to a cold protein solution while stirring forces the protein out of the solution.

Heat coagulation can also be used as a concentration step; however, the protein denatures and subsequent functional use is limited. Thus, heat coagulation is employed when nutrition and digestibility are the only required properties. Heat coagulation can also be used for protein fractionation profiting the

differential denaturation temperatures of the various proteins present in the raw material, although this technique is not currently used commercially for protein isolation.

Following these steps, a protein preparation of a various purification degrees (meals, concentrates and isolates; e.g. Table 15.2 for soybean) is achieved. Further purification by chromatographic techniques will render a high purity protein preparation and is mostly used for high value proteins required in relatively small quantities, thus, it is mainly used by the biopharmaceutical industry. Finally, the isolate is dried. This is a critical step in which temperature must be controlled to circumvent protein denaturation. Most vicilin-like globulins denature at temperatures around 65 °C. Higher temperatures are generally acceptable for legumin-like globulins and cereal proteins. The most commonly used drying methods are drum drying and spray drying (usually in-line with a fluid bed dryer). Freeze drying is only of interest for some special products. In drum drying the protein solution is applied in a thin coating to a hot surface, causing a large amount of water to evaporate within a few seconds. In spray drying the protein solution is atomized and dried with hot air. Spray drying is the most common industrial process to produce thermally undamaged protein preparations, although some irreversible aggregation may occur.

15.4.1 Seed protein extraction

Proteins are not the major component of seeds and they usually appear linked to other components such as cell wall material or starch granules. Many industrial processes exist for the extraction and concentration of proteins from seeds due to their diverse chemical nature and structure. However, certain steps are of common use unless they are demonstrated to be irrelevant for a particular seed source. Figure 15.4 displays an overview of the main steps for seed protein isolation. Seed coats and hulls are generally removed before extraction as they often contain undesirable compounds such as pigments, phenolic compounds or fibres. For many oilseeds such as soybean, cottonseed and sunflower, hulls can be easily removed, but it is more difficult for smaller seeds, such as rapeseed since the hulls tend to adhere to the endosperm. Seed are also usually milled prior to liquid extraction. This is an important step as a coarse fragmentation of the seed may impair protein extractability. The regular granulometry of the crushed or milled seeds influences the reproducibility of the protein extraction. The water content of the seed also influences the granulometry and all material should be kept at constant moisture before grinding.

Protein enriched fractions are often obtained by direct air-classification of milled seed flours, mainly for cereals, legumes and defatted meals from oilseeds, as a high lipid content may result in agglomeration. Air-classification is a dry processing method that blows away the lighter particle (as starch granules), thus removing them from the protein. This technique may render high native protein content flours. Air-classification is commonly used to produce pea protein concentrates (~50% protein content on dry basis) from pea flour. Pea protein isolates (~85% protein content on dry basis), instead, undergo a wet processing in which the extracted proteins are subsequently isolated by selective precipitation at the isoelectric point.

The bulk of oilseeds are still primarily processed for oil. One of the by-products of the oil extraction process is the oilseed meal which has high protein content (35–60%). During oil production protein denaturation occurs due to mechanical pressing and solvent extraction at elevated temperatures, resulting in an insoluble and poorly functional protein fraction. Sunflower seed processing (Fig. 15.5) is a representative example for oilseeds. Two main methods are used. These are the full press method (screw press or expeller method) and the pre-press solvent extraction. Prior to pressing, the seeds are usually partially (70%) dehulled, ground, rolled and heated. Heating facilitates the disruption of tissues, coagulates the protein, inactivates enzymes (such as phospholipases and lipases), increases the fluidity of the oil, eliminates moulds and bacteria, and dries the seed to a suitable moisture content.

The pre-press solvent extraction is the most common method for sunflower oil extraction. In this method, the seeds are screw-pressed to obtain oil and a cake with an oil content of about 16% (w/w). The cake obtained is subsequently granulated or flaked and the oil extracted with a solvent, usually hexane. In addition to the main methods, the oil can also be obtained by direct solvent extraction. In this method, the kernels are conditioned and flaked, and oil is extracted directly by solvent. This last method is the process commonly used for soybeans. Finally, the solvent is removed from the meal in a desolventizer-toaster (DT) or in a flash desolventizer system (FDS). The heat treatment in a DT is quite insensitive and results in darker meals, due to Maillard compounds (reaction of sugars with lysine and other amino acids), with a low solubility. Heat treatment in FDS systems is milder and easier to control obtaining whiter meals with higher solubility. The latter meals are preferred for food applications.

Defatting of cereal flours prior to extraction is sometimes advised in order to avoid the formation of lipid-protein complexes, although this may result in some protein denaturation and the loss of some

protein components (e.g. lipothionines). Due to the intrinsic poor solubility of cereal proteins, extraction is carried out with aqueous solutions containing alcohols (mainly ethanol and propanol), surfactants, reducing agents (mercaptoethanol, dithiothreitol), weak acids (generally acetic and lactic acid) and urea.

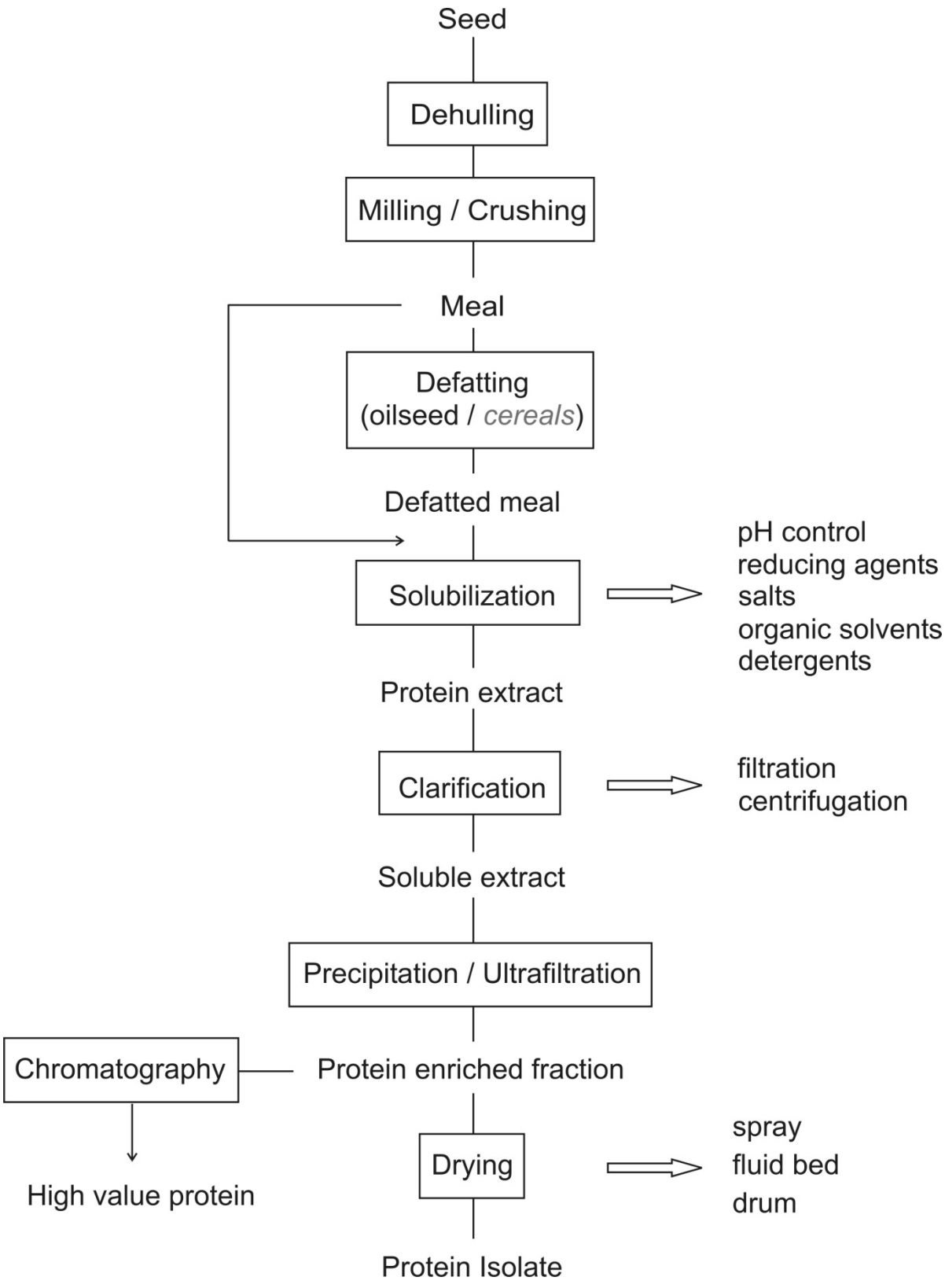

Fig. 15.4 Main processing operations to prepare a vegetable protein isolate.

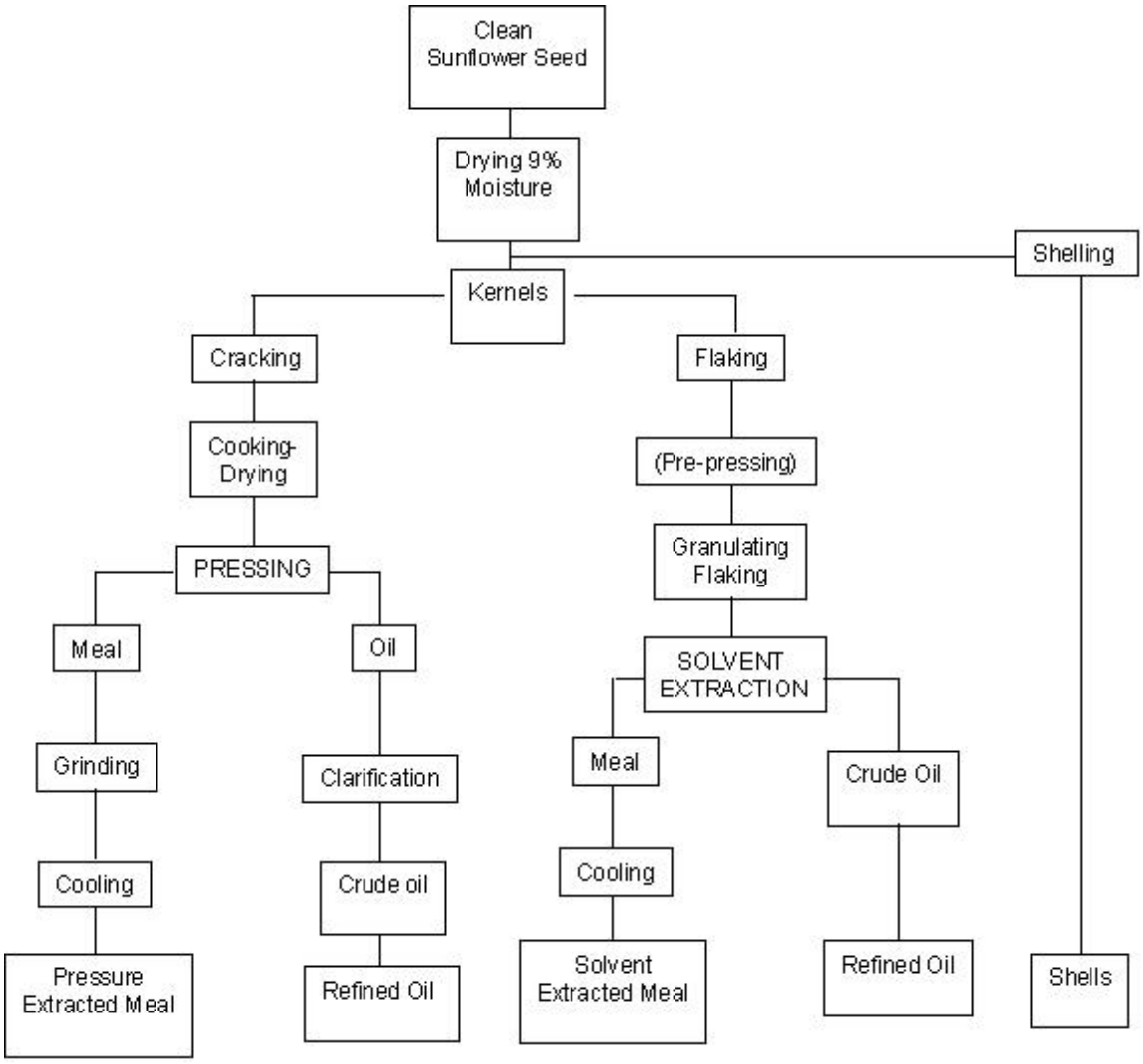

Fig. 15.5 Sunflower oil and defatted meal manufacture scheme.

15.4.2 Potato and green leaves processing

Potato tuber proteins are obtained in the potato juice, a by-product of starch manufacture. The process starts washing the potatoes prior to grinding (rasping machine) in the presence of sodium bisulphite to avoid oxidation of phenolic compounds. The rasped potatoes are then passed through rotating sieves that retain the potato pulp (potato fibres). The starch slurry is further processed in continuous centrifugal separators or hydrocyclones and sieves. The resulting potato juice contains approximately 1.5% (w/v) soluble protein.

The basic steps for the production of LPC are plant leave grinding and juice separation by pressing. The protein is dissolved in the juice, after which it is coagulated, usually by heating, and then dried.

15.5 Technical data: functional properties

The first functional protein preparations for food application were dehydrated natural products such as milk or eggs. The first protein replacer in Europe—a milk powder hydrolysate, marketed as egg-white substitute—was produced during the Second World War. Nowadays, protein preparations for food use are extracted from plants, animals and microorganisms (single cell protein, SCP). However, the successful use of proteins for food applications depends on their functional properties rather than on their nutritional

quality. Functional properties refer to the overall behaviour of proteins in food and reflect the various interactions in which proteins take part. Functional properties are related to the physical, chemical and conformational properties of proteins in food systems during processing, storage, cooking and consumption. The primary characteristics that determine the functional properties of proteins in food systems include molecular weight, size, shape, flexibility, amino acid composition, structure, net charge, charge distribution and hydrophobicity. Functionality may vary with the source of protein, its composition, the method of preparation, its thermal history and the prevailing environment (i.e. pH, ionic strength, temperature, presence of salts, etc.).

There is a strong correlation between structure and functional properties of proteins in food systems. The knowledge about this correlation is essential for the optimal use of proteins in food systems. However, establishing the structure-functionality relationship for food proteins is not trivial because of the complexity of vegetable food products. Therefore, of utmost importance is a basic understanding of protein structure, solubility and stability. Protein structure has been described in Section 15.3.

15.5.1 Protein solubility and stability

Solubility of a pure substance is the amount of substance in solution in equilibrium with its crystalline form. For industrial applications, a practical definition of solubility is the amount of protein that goes into solution or colloidal dispersion under specific conditions (pH, ionic strength, etc.) and is not sedimented by moderate centrifugal forces. Proteins vary enormously in their solubility: some vegetable globular proteins (i.e. albumins and globulins) are very soluble while proteins involved in building structural elements in organisms (i.e. membrane proteins) are essentially insoluble. Vegetable protein preparations often contain phenolic compounds that tend to decrease solubility. In general, the more polar its surface is, the more soluble a protein is likely to be, since interactions with solvent molecules principally involve amino acid residues at the protein surface.

Solubility is considered a key functional property since good solubility is a prerequisite for the success of protein preparations in most functional applications. These properties will determine the field of application as well as whether a new protein ingredient will be competitive on the market. If such properties are lacking, the preparations will almost only be used as animal feed and food fortification.

The net stability of the folded (native) state of a protein depends upon a complex balance between the many diverse interactions present in the folded state, the higher conformational disorder of the unfolded state and the interactions with the solvent. The folded state is easily disrupted by environmental conditions often occurring during food processing (e.g. extreme pH values, pressure and temperature, organic solvents, etc.). Denatured proteins are unfolded but do not undergo changes in their covalent structure with the possible exception of breaking and reshuffling of disulphide bonds. Unfolding of proteins has been reported to improve protein functionality of sunflower, potato and soybean proteins. However, the positive effects of protein unfolding are usually counteracted by protein losses due to precipitation.

15.6 Functional properties for industrial applications

Proteins are expected to contribute to the product consistency and juiciness due to their gelling, texturizing, dough forming, water- and fat-holding capacities. Other desired properties are the formation and stability of foams and emulsions.

15.6.1 Foams and emulsions

Foams and emulsions are colloidal systems in which one phase (air for foams and oil for oil/water emulsions) is dispersed in another phase. For the formation and stabilization of foams and emulsions, the adsorption of proteins and subsequent lowering of the surface tension, onto air/water and oil/water interfaces, respectively, is essential. Molecular properties such as conformational stability/flexibility, surface hydrophobicity, and effective molar mass govern the ability of proteins to lower the interfacial tension during foam and emulsion formation, hence, facilitating the formation of small particles. Although foams and emulsions are both dispersed systems and the processes that occur in the formation and stabilization are similar, gas bubbles are larger and more susceptible to disturbing influences (i.e. temperature gradients, dust, evaporation, etc.) than emulsion droplets.

Emulsions

Proteins are suitable to make oil/water emulsions, but not water/oil emulsions, as described by Bancroft's rule: the phase of an emulsion in which the surfactant (protein) is more soluble becomes the continuous phase upon agitation of the mixture. To make an emulsion, it is necessary to apply a substantial mechanical energy. In most situations the drops are formed by intense agitation, homogenization, rapid blending, stirring or cutting. The smaller the droplet, the more stable the emulsion is. The most important factor during emulsion formation is the effective molar mass of the protein: the larger it is, the higher the amount of protein required to obtain small droplets. However, at high protein concentrations, there are no significant differences among proteins regarding their ability to form small droplets. Once formed, the emulsion is exposed to various physical destabilizing mechanisms, such as creaming, aggregation (flocculation) and coalescence (Fig. 15.6).

Foams

The most common methods to make foams are: (i) via supersaturation under pressure, followed by pressure release, (ii) by gas injection into a liquid; and (iii) by beating in air. The latter is the most commom method used by the food industry. Faster beating often results in higher foam volumes and smaller bubbles.

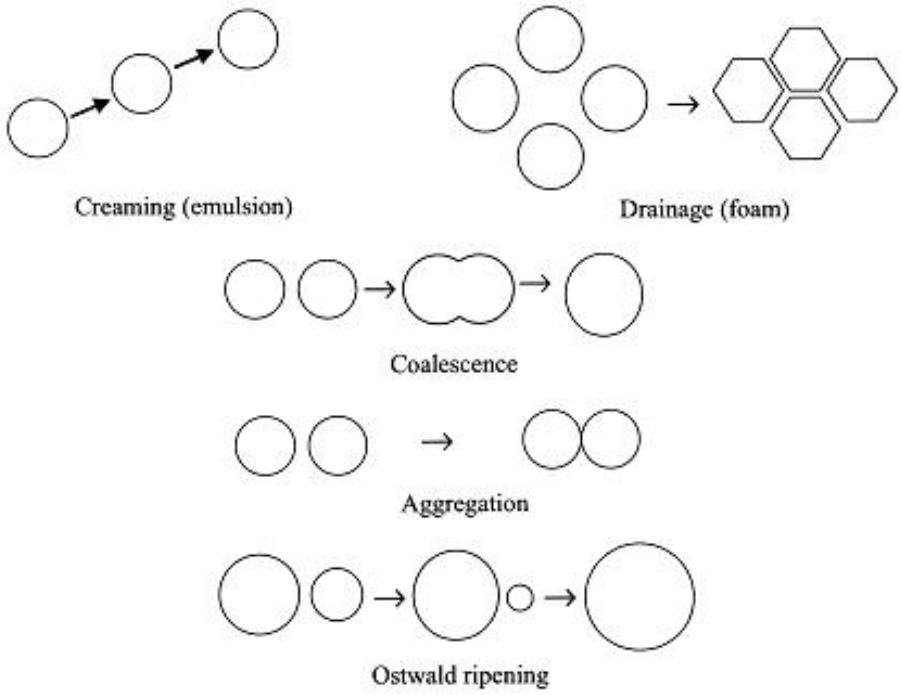

Fig. 15.6 Instability mechanisms of foams and emulsions.

Proteins are suitable surfactants to make foams, although there are large differences in the foaming ability among proteins, in particular for foams made by beating. This is mainly because at short time scales some proteins (e.g. proteins with high conformational stability) can hardly adsorb to the air/water interface. The main functions of proteins in foams are to decrease interfacial tension, to increase viscous and elastic properties of the liquid and to form strong films. Physical destabilizing mechanisms of foams concern changes in position or size of the bubbles such as drainage, coalescence and Ostwald ripening (Fig. 15.6). Ostwald ripening, the growing of large bubbles at the expense of smaller ones, is the most important type of instability in foams.

15.6.2 Gels and related aspects

Proteins are generally not suitable as thickening agents, due to the high concentration required to achieve an adequate viscosity. However, they can be used to make gels. Protein gelation is used in numerous

industrial applications. Gelatine is certainly the most widely used gelling agent; however, as mentioned above, there is an increasing demand for vegetable-based products.

A gel consists of a matrix (three-dimensional network of connected macromolecules or particles) and a continuous liquid phase, mechanically retained by the matrix. A gel is a material with predominant solid-like characteristics but it deforms under stress. Gel formation is a complex process that often involves several steps such as denaturation, aggregation and network formation (Fig. 15.7). Protein denaturation preceding gel formation is generally accomplished by heating. Heat-induced gelation is one of the most commonly studied phenomena in food science and responsible for the structure of many food products. Gelation can also be induced by high hydrostatic pressure, cold gelation (salt-induced and acid-induced), and enzyme-induced gelation. During denaturation the protein adopts an unfolded conformation in which functional groups become exposed. Consequently, these exposed groups can interact with each other to form aggregates that may precipitate at low protein concentration and may gel at high protein concentration.

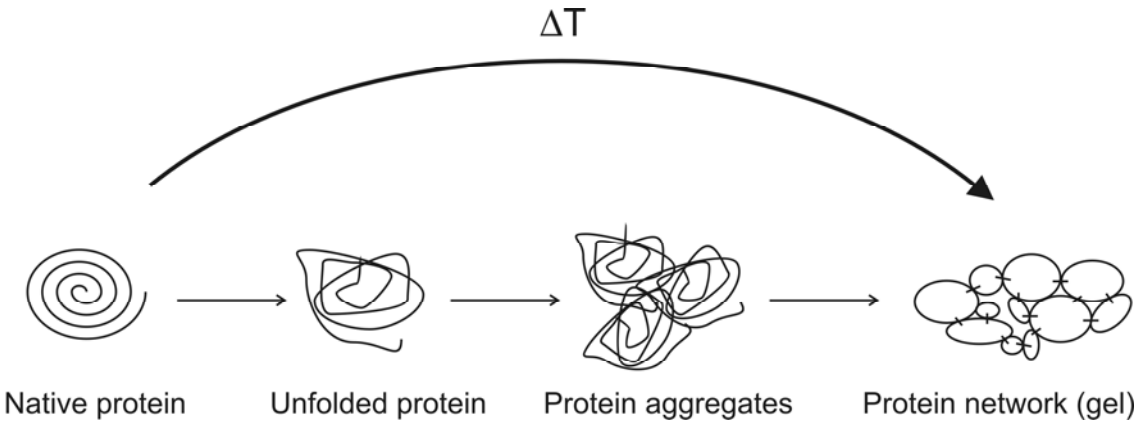

Fig. 15.7 Heat-induced gel formation.

For globular proteins two main types of gel networks can be distinguished according to microscopic observations: fine-stranded and coarse networks. In fine-stranded gels the proteins are attached to each other like a string of beads. This type of gel is generally transparent. Coarse gels are non-transparent and are formed by random aggregation of proteins into clusters with diameters in the range of 100–1,000 times a single protein molecule. The type of gel that is formed depends on conditions during gel formation, generally gels are coarser as the pH value approaches the isoelectric point and when the ionic strength is increased. Network structure affects gel properties, including permeability, the ability to retain water and rheological properties.

15.6.3 Texturization of proteins

Texturization of proteins can be understood as the creation of a three-dimensional structure to substitute partially or totally other proteins (meat analogues) or lipids (fat substitutes) in traditional foodstuffs. Typical structures are fibres, shreds, chunks, bits, slices, films and granules. Protein texturization generally involves a first denaturation step followed by the re-organization and orientation of the partially or totally unfolded proteins by extrusion, rolling/ spreading or surface precipitation. The final step is the binding and stiffening of the organized protein structure. Many texturizing methods are available for the food industry such as extrusion cooking, spinning (dry, wet and spinneretless), freeze texturization, high pressure texturization, and chemical or enzymatic texturization. Because of the numerous processes and products obtained, this point will not be described further.

15.6.4 Water- and fat-holding capacity

The interaction of water with proteins is very important both to the structure of the proteins and to their behaviour in food systems. Water-holding capacity is generally defined as the ability of a protein matrix to absorb and retain bound, hydrodynamic, capillary and physically entrapped water against gravity. Chief factors affecting water binding by food proteins are amino acid composition, protein conformation, surface

hydrophobicity, ionic concentration and pH. For instance, proteins that contain large amounts of the charged amino acids tend to bind large amounts of water.

The capacity of a protein to bind lipids is an important characteristic to enhance flavour retention and to improve mouthfeel for such applications as meat substitutes and extenders. Protein isolates can be added to ground meats to promote fat binding and decrease cooking losses since fat separation is a common problem in processed meat products.

15.6.5 Dough formation

Dough is an extensible, viscoelastic protein network formed upon the mixing of water and cereal proteins. Mixing of dough is considered a critical step for bread quality. During wheat dough mixing, gliadins and glutenins unfold exposing hydrophobic groups which cause the proteins to aggregate and to crosslink due to the formation of disulphide bonds. The latter results in a viscoelastic network structure surrounding the starch granules and able to expand when gas is formed by the metabolism of yeast or by the water vapour expansion during heating.

15.7 Chemical and enzymatic modification of protein products

Proteins can be modified to alter specifically their physico-chemical and functional properties. Protein modifications can be defined as irreversible changes in the protein structure by physical, genetic (i.e. GMO), chemical and enzymatic treatments. In this chapter, the last two methods are described briefly.

15.7.1 Enzymatic modification

A broad range of post-translational modifications occur naturally in the cell. Most of them are catalysed by enzymes. However, only two enzymatic modifications are of general commercial interest: crosslinking of proteins using transglutaminase and proteolysis.

Transglutaminase catalyses the formation of a covalent bond between gamma-carboxylamide groups of peptide- or protein-bound glutamine residues and primary amino groups, resulting in inter- and intramolecular crosslinks. At functional level this reaction improves viscosity, texture, water-holding capacity and stability (regarding temperature, syneresis and emulsification). However, it may decrease emulsion and foam formation due to the lower protein flexibility. Furthermore, it may hamper gel formation since the gelation temperature is higher. The main current application is in the production of reformed meat and improved sausage texture. Nowadays, many patents have developed regarding transglutaminase crosslinking in food products such as bakery products, noodles and pasta, from soybean and other vegetable proteins.

Proteolysis is conducted in many food industries with various aims such as food flavouring, functionality or pharmaceutical applications. Proteolysis results in decreased molecular weight, increased charge (more terminal residues), and more random structures, including exposed hydrophobic residues. From a functional point of view, there is an obvious improvement of solubility and a higher ability to form foams and emulsions, although generally with decreased stability. Regarding gelation, proteolysis usually results in poor gelling properties. Nutritional properties of hydrolysates reflect their increased digestibility and decreased allergenicity compared to the parental proteins. Besides nutritional aspects, many peptides may exhibit specific physiological properties, such as immunomodulatory effects or antimicrobial activity. Bioactive peptides can be released during protein hydrolysis. Several bioactive peptides have been found in protein hydrolysates, as opioid, anti-hypertensive, and anti-thrombotic peptides. For instance, the peptic hydrolysates of wheat gluten and rice prolamin have been shown to possess an opioid-like activity.

A wide range of commercial proteases are available, being the cheapest the food grade versions of detergent enzymes (e.g. alkaline bacterial proteases). Other sources include plant enzymes such as bromelain, animal enzymes such as chymotrypsin, and fungal (*Aspergillus*) proteases.

15.7.2 Chemical modifications

Chemical modifications are generally carried out under more extreme conditions (pH, temperature, solvent, etc.) and often lead to high heterogeneous products with poor specificity; therefore, legally there is a more restricted allowance for food applications. Furthermore, there are concerns regarding toxicity, loss of nutritional value and worsening of organoleptic properties. Two chemical modifications are commercially used: deamidation and hydrolysis. Hydrolysis is used for preparing hydrolysed vegetable protein mainly as flavour enhancer. Deamidation of carboxamide groups leads to a marked decrease of the

isoelectric pH and increased negative charge. Levels of deamidation as low as 2–6% can enhance the functional properties of proteins. Deamidation of gluten proteins have been performed resulting in improved solubility, emulsifying and gelling properties.

Many other chemical modifications have been studied to enhance functionality of vegetable proteins, but the low specificity and complex regulatory issues regarding food applications have limited their use. Most promising are glycosylation, succinylation, phosphorylation, alkylation and redox reactions. For instance, the addition of redox reagents to flour and bakery products has been a common application.

15.8 Vegetable proteins: choosing the best functionality for the application

The large variety of vegetable protein sources provides the food industry with a broad choice of protein sources to obtain the desired textural, sensory and nutritional properties. Table 15.3 provides a general overview of the key functional properties of proteins required for various food applications and the most common vegetable protein sources used. However, it should be emphasized that the physico-chemical properties, and thus the functional performance of proteins in foods, vary extensively with the operating conditions and industrial process used for manufacturing the protein preparations. For instance, drum-dried pea isolates have higher fat-holding capacity than spray-dried pea isolates, but lower foaming, colour and flavour properties.

Soybean protein preparations are by far the most common vegetable source of proteins as functional food ingredient. Comparison of other vegetable protein sources with soybean is frequently found throughout the literature because the extensive research performed on soybean protein functionality is a useful base resource to take advantage of. However, the numerous publications on vegetable proteins provide a great variety of results, sometimes conflicting, regarding functional properties. Therefore, they do not often permit general statements on the suitability of specific vegetable proteins for specific applications. The variety of results is likely due to the diversity of tests described in the literature to assess protein functionality, as well as to the array of methods to obtain the protein preparations. The latter results in products that differ in:

- protein content and protein composition (e.g. ratio albumin/globulins)
- presence of non-protein compounds such as pectins, fibres, and phenolic compounds that influence the functionality of the system
- degree of protein denaturation
- the variety and cultivar used.

In conclusion, choosing the most suitable vegetable protein source for an application requires good knowledge of both the preparation method for the specific protein and its processing history.

15.9 Applications of vegetable proteins in food products

Vegetable proteins have been used for a number of reasons, besides the obvious nutritional value in foods; to provide specific required properties and structural basis to the products in which they are added. With regard to desirable properties provided to food systems, proteins are generally expected to ensure physical stability (avoiding or delaying sedimentation, oil separation, coarsening, etc.) and attractive usage properties such as desirable texture, consistency or viscosity. An example that illustrates the latter is the number of imitations of meat products with vegetable proteins that not only have eating characteristics, such as texture, colour or flavour, similar to meat, but also an adequate nutritional profile.

The use of vegetable proteins for novel food applications requires an understanding of the complex nature of food systems. Most food systems are heterogeneous regarding:

- protein composition, as it concerns generally mixtures of various proteins,
- water, lipids, carbohydrates, and other minor components such as salts or pigments; some of them can markedly interact with proteins and affect the organoleptic and nutritional characteristics of the food product, and
- structural organization (emulsions, foams, films, etc.).

Moreover, proteins are not often completely native. They can be totally or partially denatured or may have undergone other modifications, such as partial proteolysis.

15.9.1 Food applications of seed protein preparations

Over recent years, vegetable-isolated proteins from seeds have become crucial market commodities for imparting functionality and texture as well as for enhancing nutritional value in a variety of food products. However, existing seed protein containing foods from non-animal protein sources are still considered to have inferior flavour and texture.

Table 15.3 Required functional properties of vegetable proteins for food applications

Food application	Property required	Protein requirement/ mechanism	Main Protein sources
Bakery	Viscosity, elasticity, gelation, water binding	Hydrophobicity, disulphide crosslinks, network formation, hydrogen bonding	Cereals
Low-fat bakery products, doughnuts	Fat and flavour binding	Hydrophobic bonding entrapment	Cereals
Desserts, dressings	Solubility, emulsifying/ foaming properties, fat mimetic	Hydrophilicity, molecular flexibility, interfacial adsorption	Soybean, peanut, lupin, other legumes and oilseeds
Dairy substitutes	Solubility, colour–free, tasteless, emulsifying properties, stability to heat	Hydrophilicity, molecular flexibility, interfacial adsorption	Soybean, peanut, lupin, pea, other legumes and oilseeds
Fortification	High nutritional value, solubility	Digestibility, hydrophilicity absence of allergens	Amino acid balanced vegetable protein mixtures, hydrolysates, nutritional quality improved by fermentation and germination
Infant formula	High nutritional value, solubility, emulsifying properties, stability to heat	High digestibility, fully absence of allergens, hydrophilicity, interfacial adsorption	Soybean, vegetable protein hydrolysates
Meats and sausages substitutes	Texturisation, solubility, emulsifying properties, water binding, fat mimetic, gelation	Hydrophilicity, , network formation, water entrapment and immobilization, interfacial adsorption, disulphide crosslinks	Soybean, pea, lupin, wheat
Beverages, soups, gravies	Solubility, viscosity, acid stability	Hydrophilicity, protein solvation	Soybean, pea, vegetable protein hydrolysates, fermented cereals and legumes
Cheese-like products	Gelation, solubility, colour–free, tasteless	Hydrophilicity, protein solvation, network formation, water entrapment and immobilization	Soybean

Table 15.4 Food applications of the main soybean protein preparations

	Bakery	Dairy products/desserts	Meat substitutes	Other products
Flours and grits	Bread, cakes, biscuits, pancakes, pasta, doughnuts	Milk substitutes	Bolognese sauce, meat balls and hamburgers, patties	Snacks, candies, dietary products
Concentrates	Bread, cakes, biscuits, pancakes, pasta, doughnuts	Milk substitutes, cheese, coffee whiteners, frozen desserts, infant formulas	Sausages, Bolognese sauce, meat balls and hamburgers, seafood, patties	Snacks, candies, dietary products, soups, gravies and beverages
Isolates	Bread, cakes, biscuits, pancakes, pasta	Milk substitutes, infant formulas, cheese, coffee whiteners, frozen desserts	Sausages, Bolognese sauce, meat balls and hamburgers, seafood, patties	Snacks, candies, dietary products, soups, gravies and beverages
Texturised protein	No generally used	No generally used	Bolognese sauce, meat balls and hamburgers, seafood (e.g.surimi), patties, muscle-like products, dried meat bits, stews	Snacks, soups and gravies

The largest market for protein preparation in human foods continues to be soybean (Table 15.4), although protein isolates from other grains, e.g. pea, wheat, lupin, sunflower, cottonseed or rapeseed, are currently being explored with great interest. The consumption patterns of soybean are not homogeneous around the world. In the Far East, soybeans have been consumed for thousand of years in various forms in traditional foods such as tofu, soymilk and fermented products. In the Western world, it is only during the last 40 years that soybean products have been more widely consumed; mostly in the form of refined soybean protein ingredients used by the food industry. However, an estimated 85% of the world's soybeans are converted into oil and defatted meal. The meal is mainly used in animal feed. A small portion is further processed into food ingredients including soybean flour, concentrates, isolates, hydrolysates and textured proteins.

A key limitation of soybean has been the characteristic beany and greeny flavour in soybean products associated with the presence of lipoxygenases. This flavour is particularly problematic to western consumers, who are not familiar with it. Lipoxygenases oxidizes carotenoids and chlorophyll in flour to their colourless form. It can also oxidize fatty acids with the subsequent development of rancidity. A further constraint is the presence of several anti-nutritional factors. However, the latter problems are mostly overcome by applying the proper treatment (e.g. heating).

Nowadays, a broad choice of soybean protein preparation is available for the food market: roasted soybean nuts, full-fat soybean flour and grits (three types: enzyme-active, heat-treated, extruder-processed), enzyme-inactive low-fat soybean flour or grits, enzyme-active defatted flake/soybean flour (of several protein dispersibility index; PDI), lecithinated soybean flour, textured soybean flour, refatted soybean flour, soybean concentrates, textured soybean concentrates, soybean isolates, and organic soybean flour and concentrates. These soybean protein preparations are applied as ingredients in numerous food products as functional and nutritional substitutes for meat, milk and egg protein. There are many textured soybean products, mainly used as meat substitutes, where the chief advantages are reduced lipid content and increased protein levels. Available textured products are marketed in several colours, allowing a variety of applications in chicken, beef, pork or mutton-style dishes. Normally, the meat substitute is dried after extrusion, resulting in a very shelf-stable product. The most important characteristics of the meat substitute are their meat-like appearance, texture and cooking properties. They can be prepared by steaming, barbecuing, frying or stewing, as an original meat product.

Other soybean markets include bread and bakery to enhance the nutritional value of the cereal protein by supplying lysine to the formulation. Soybean proteins also enhance the shelf-life of bakery products thanks to their ability to retain water. Other food applications include dietetic and health foods, beverages, dairy analogues (soymilk, soywhey, tofu, whipped toppings, and ice-creams), breakfast and infant formulae. Figure 15.8 depicts the standard process to prepare soymilk, tofu, soybean whey, and okara (soy pulp).

Peas have low protein content compared to soybeans; pea meal consists mainly of starch and generally is not defatted prior to protein extraction. Therefore, lipid solvent extraction and the subsequent heat treatment to remove the solvent may be avoided. Furthermore, the analogy between globular proteins in pea and soybean means that the extensive functional and technological knowledge already existing for soybean protein products can be exploited. Textured pea protein is finding wide application in vegetarian foods such as burgers and sausages. In recent years, ingredients from pea protein preparations with brand names, such as Arrum (made from yellow peas and wheat gluten in a 1:1 ratio), have been used to formulate new products. Arrum resembles chunks of meat and is suitable for burgers, pies and lasagne. More common uses are in pea snacks, such as fried peas coated with wheat and/or rice flour, or extruded fried green pea flour products flavoured with seasonings.

There is increasing technological and research interest into the development of protocols for preparing legume ingredients and food items accepted by consumers for their sensory and nutritional characteristics. For instance, various protein lupin products are already available in the market for the food industry: toasted and non-toasted flour, grits, granulates, and protein concentrates from non-defatted seed. These lupin protein products have already been developed in different sectors like bakery goods (muffins, biscuits), emulsified foods (drinks, imitation cream, dressings), and extruded foods (pasta, snacks).

Cereals are processed by the food industry to prepare a broad choice of products:

- baked products, from flour or meal, as pastries, pancakes, biscuits, and cakes
- milled grain products, made by removing the bran and usually the germ such as rice, enriched flour, wheat flour, bread flour, durum flour, couscous, grits, hominy, cornmeal, pearled barley, pot barley, semolina, prepared breakfast cereals, soup, gravy, and other thickenings

- on the drinks side, cereals are used for brewing and distilling alcoholic drinks thanks to their high carbohydrate content
- whole-grain products include rolled oats, brown rice, popcorn, shredded and puffed grains, and breakfast foods.

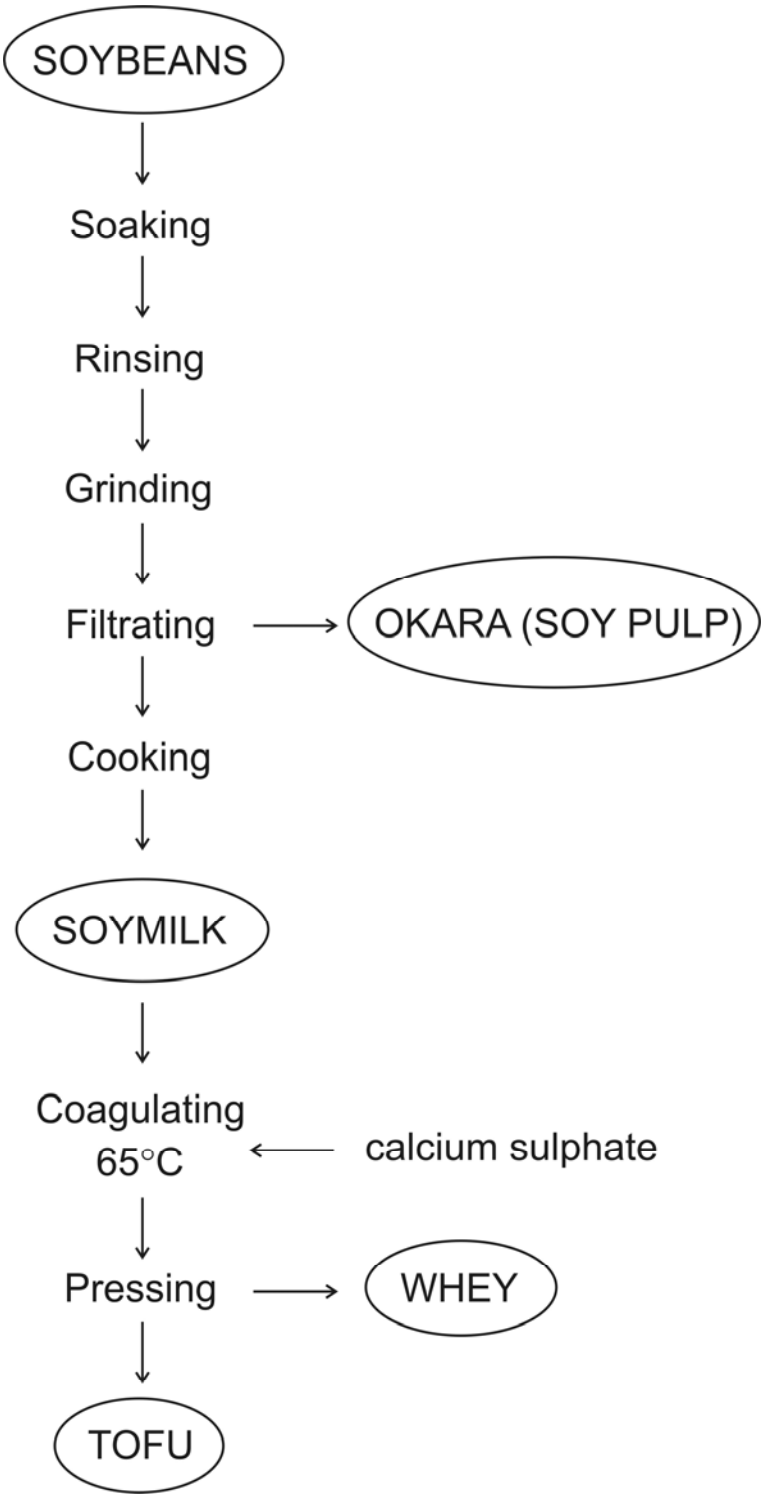

Fig. 15.8 Production of soymilk, soywhey, tofu and okara.

Bread making is the largest market for cereals, employing about 64% of the wheat world production. A relatively recent application of wheat is its use as meat substitute marketed as 'Wheatpro' in the early 1990s. Gluten is processed and extruded to resemble the texture of meat.

Oilseeds can be carefully processed or refined for food use. Some progress has been made for sunflower, peanuts, rapeseed, cottonseed and sesame. However, oilseed protein-based products are still marginally developed. There are some applications to fortify foods (as infant formulae, bakery or pasta products) or milk substitutes (e.g. peanut milk). The use of other oilseed proteins, such as sunflower or rapeseed, as dairy product analogues has been investigated. Fortified dairy products are prepared by blending pasteurized whole milk, dried skim milk, and oilseed meal or protein isolates. However, the successful production of oilseed protein-based dairy products requires protein isolates with significantly improved flavour, colour and functional properties. The need for such high quality protein products stems from the mild flavour and soft colour of most dairy products.

15.9.2 Food applications of potato and green leaves

Food applications of potato proteins are still constrained by the lack on an efficient non-denaturing large-scale method for their recovery. Furthermore, the presence of protease inhibitors is undesirable in foods. Research efforts are aimed at inactivation procedures that avoid extensive protein insolubilization. Potato proteins can be used for fortifying bakery products. In normal bread dough the possibility of addition of potato protein is limited, while the protein content in crispbread can be doubled without essential changes in its typical characteristics, such as crumb structure, specific volume and hardness.

LPC has been examined as a human food source, because it is potentially the cheapest, most abundant source of available protein. However, so far, due to the extensive denaturation during its preparation, LPC has been used as a nutritionally rich ingredient rather than as a functional ingredient.

15.10 Nutritional and health effects

Meat and fish products are the most readily available sources of complete protein. The protein content, by weight, of cooked animal products ranges from 15 to 40%. The protein content of cooked cereals and legumes is about 3–10%. Potatoes, fruits and leafy green vegetables come in at 3% or lower. An important aspect that greatly influences protein bioavailability and digestibility is the processing history of the protein products, which also has a direct effect on the functional performance of the proteins.

Vegetable protein sources often contain natural occurring anti-nutritional factors that hamper the nutritional potential by interfering with the intake, bioavailability or metabolism of nutrients. Table 15.5 shows the most common anti-nutritional factors that can be found in vegetable protein sources.

The presence of high levels of protease inhibitors from legumes can cause considerable reductions in protein digestibility. Similarly, the presence of high levels of tannins in cereals and legumes can result in significantly reduced protein bioavailability. To improve the nutritional value of the vegetable proteins, several strategies have been developed. Most often used are seed dehulling, soaking, thermal treatments, irradiation and protein fractionation. Other approaches are based on breeding, hydrolysis, germination and fermentation.

Anti-nutritional factors can also be formed during food processing by heating/alkali treatments, organic solvents, oxidizing agents or acids. For instance Maillard compounds, oxidized forms of sulphur amino acids (such as methionine sulphoxide, methionine sulphone, and cysteic acid), D-amino acids, and crosslinked amino acids such as lysinoalanine and lanthionine.

15.10.1 Legumes

Leguminous species are used as dry grains in appreciable amounts for human nutrition. Because of the balanced proportion of proteins (except for sulphur-containing amino acids), starches, fibres and minerals, legume seeds complement cereals in human diets. They are also good sources of minerals and vitamins. Germinated legumes are rich in vitamin C, riboflavin and niacin. Several studies claim that the consumption of legumes has interesting physiological effects, preventing some common health problems, such as diabetes mellitus, coronary heart disease and colon cancer. Therefore, legume protein preparations are gaining importance in the food industry, representing an attractive alternative in the development of new foods.

Table 15.5 Antinutritional factors of vegetable protein sources

Antinutritional factor	Health effect	Main vegetable source
Protease inhibitors	Digestion impairment	Legumes, potato, peanut, soybean, tomatoes
Gossypol	Heart and liver toxicity, weight loss, and depressed appetite	Cottonseed
Hemagglutinins and lectins	Interaction with gastrointestinal mucosa, vomiting and diarrhoea	Faba bean, pea, peanut, soybean, castor bean
Mycotoxins	Liver damage	Cottonseed, peanut, maize, rice
Cyanogenic glycosides	Headache, muscle weakness, nausea, etc.	Almond, cassava, sorghum, lima beans
Phytic acid	Reduced Ca and Fe absorption	Oilseeds, cereals, soybean
Tannins	Reduced Ca and Fe absorption, gastrointestinal disturbances	Legumes, cereals, faba bean
Oligosaccharides	Flatulence	Legumes
Oxalates	Reduce Ca absorption and encourage kidney stone formation	Sesame, spinach
Oxaldiaminopropionic acid (ODAP)	Neurological lesions (lathyrism)	Grass pea
Goitrogenic factors	Interfere with iodine uptake, goitre	Rapeseed, mustard seed, cassava, sorghum, broccoli
Favism causing compounds (e.g. vicine)	Fatigue and nausea for people carrying the hereditary disorder	Faba bean, pea

15.10.2 Cereals

All cereal grains have high energy value, mainly from the starch fraction but also from the fat and protein. Cereals are rich in fibre, B vitamins and minerals. However, during processing many of the nutrients are lost. In general, the cereals are low in protein content, although oats and millets are exceptions. Cereal proteins are characterized by the high content of glutamic acid and proline, being generally deficient in lysine. Rice and oat proteins are richer in lysine and are often considered to be of better nutritional quality. In contrast, wheat and maize have the lowest lysine content. Tryptophan is the second limiting amino acid in cereals, particularly in maize. Other cereals, such as quinoa, buckwheat and grain amaranth have a well-balanced essential amino acid profile. Quinoa contains up to 18% protein, being an unusual complete vegetable source of protein.

15.10.3 Oilseeds

Oilseeds are energy dense foods, due to their high oil content. They are a source of fibre, vitamins (vitamin E, niacin, and foliate), minerals (phosphorus, iron, and magnesium), monounsaturated (e.g. peanuts) and polyunsaturated (e.g. sunflower) fatty acids. Oilseed proteins are deficient in lysine and sulphur amino acids compared to animal proteins and may contain anti-nutritional factors. Despite these limitations, easily overcome by supplementation with other proteins and physico-chemical treatments respectively, oilseed protein makes a significant contribution to the human dietary protein intake. Furthermore, oilseeds are generally richer in sulphur amino acids than legumes, but poorer in lysine except for rapeseed. The nutritional quality of oilseed meals depends largely on the oil extraction process. Severe heat treatment results in the destruction of several amino acids, particularly lysine, methionine, arginine, tryptophan and cysteine.

15.10.4 Potato and green leaves

Potato, a carbohydrate-rich food, is a good source of vitamins C, B1, B3 and B6, and minerals such as potassium, phosphorus and magnesium. It also contains folic acid, pantothenic acid and riboflavin. As far as the amino acid composition is concerned, the quality of potato protein is fairly good, with relatively high lysine content. In contrast, it is low in methionine. Glycoalkaloids (usually solanine and chaconine) are the main anti-nutritional compounds of potatoes together with protease inhibitors.

Green leafy vegetables are rich in carotenoids as well as in iron, calcium, magnesium, vitamin C, riboflavin and folic acid. Leaf proteins are a good source of amino acids, with methionine being a limiting factor. The main nutritional problems are related to high fibre content and other anti-nutritional factors, such as phytate, cyanide and tannins.

15.10.5 Protein allergenicity

Adverse reactions to foods are becoming an increasingly important health issue. In particular, the incidence and prevalence of food allergies have grown in recent years and are estimated to affect 1–4% of individuals. There are a number of groups of foods that are responsible for most food allergies. These groups include milk, eggs, fish and shellfish, wheat, legumes, nuts, fruits and vegetables.

The majority of known class 1 food allergens (via gastro-intestinal tract) belong to two superfamilies of vegetable proteins: the prolamin and the cupin superfamilies. Proteins belonging to these superfamilies include albumins (e.g. Ara h 2 from peanuts), prolamins and globulins (e.g. vicilin allergens as Ara h 1 from peanuts). Next to these superfamilies, the papain superfamily has also been reported to represent class 1 food allergens.

The allergic reaction may be caused by a peptide of a few amino acids (linear epitopes) or it may be a motif of the protein structure (conformational epitopes). Food processing may help inactivate certain food allergens, but may produce new ones as a result of change in protein conformation. Conformational epitopes are generally more susceptible to protein denaturation, while linear epitopes are more likely to be inactivated by hydrolysis.

15.11 Regulatory status

Vegetable protein products (VPP) are defined as 'food products produced by the reduction or removal from vegetable materials of certain of the major non-protein constituents (water, oil, starch, other carbohydrates) in a manner to achieve a protein content of 40% (dry weight basis) or over'. This definition conforms to the codex general standard 174-1989 developed by the international Codex Alimentarius Commission (Joint FAO/WHO Food Standards Programme). VPP proceed from several vegetable sources and their utilization in foods is highly regulated. The VPP regulation covers important aspects such as the end product labelling, the use of genetically modified organisms (GMOs), the introduction of novel food products on the market, food allergens, and modifications of food products.

The presence of VPP in foods should be clearly indicated on the label. Food products containing VPP should be labelled in accordance with general standards developed by the international Codex Alimentarius Commission (Codex stan 1-1985; Rev. 1-1991) and directives developed by the EU food safety legislation (Directive 2000/13/EC; Directive 90/496). The ingredient statement of VPP should contain the vegetable source (e.g. soy, wheat, pea, groundnut, etc.) and a complete list of ingredients should be declared on the label in descending order of proportion excluding added vitamins and minerals. When VPP are used at low levels for functional purposes, the level of VPP is calculated on a dry weight basis in

the final product. The use of VPP as functional ingredients follows the same regulation as other functional ingredients with no required change in the name of the product. VPP can be used as protein extenders to improve the nutritional value of various food products. The protein nutritional values should be assessed according to the established methods for protein quality measurement. The complementary protein should contain a minimum percentage of certain residues (lysine, methionine, cysteine or tryptophan) if diets are deficient in those residues. Addition of L-amino acids is considered if the complementary or supplementary proteins do not give the desired increase in the nutritional contribution. Fortification of VPP with vitamins and minerals can also be a commercial practice when VPP contain anti-nutritional factors that might interfere with the bioavailability or utilization of nutrients, or when VPP are used as a suitable vehicle for fortification in regions where there is a demonstrated need for intake in certain vitamins or minerals. VPP can also be used to substitute partially or completely animal proteins in foods. The protein quality and quantity of the new product cannot be less than that of the original product; and vitamins and minerals also have to be in equivalent quantities.

The name of the substituted product should describe the true nature of the product. In cases where the VPP substitution results in a product with lower animal protein content than that required to be regarded as belonging to the same category, the name of the standardized animal foods should not be used unless properly qualified. When the animal protein is substituted completely, the name of the food should be the name of the VPP with a brief description. VPP, which are not intended to supplement or substitute animal protein foods, but are used instead as the sole protein source in products, should be safe and produced in accordance with good manufacturing practices. Before the utilization of VPP as human food, VPP need to be tested pursuant to the guidelines developed by the Codex Committee on vegetable proteins (CX-728). VPP which are produced by minor processing variants from sources commonly used as food need not to be tested thoroughly; however, novel VPP, which are processed by new techniques from commonly used sources or produced from sources not previously used as human food, require thorough testing; where a description of the preparative process, nutritional value, microbiological status, and toxicological safety of the novel VPP have to be given and examined.

Some VPP have specific codex standards. For instance, VPP prepared from soybean have different names on the label depending on the protein content (soy protein flour, 5065%; soy protein concentrate, 6590%; and soy protein isolate 90% or over). Other descriptions, including the physical form and texture of soybean products, might also be declared on the label (Codex stan 175-1989). Likewise, the name of wheat gluten or wheat protein products to be declared on the label varies depending on the viscoelasticity property of hydrated gluten. The name of wheat protein products shall be 'vital wheat gluten' if the viscoelasticity is high; 'devitalized wheat gluten' if the viscoelasticity is reduced due to denaturation, or 'solubilized wheat gluten' if the viscoelasticity is reduced due to partial hydrolysis (Codex stan 163-1987).

Genetically modified plant varieties have become part of the global food market and all appropriate measures have to be taken to avoid adverse effects on human health and the environment. Before placing genetically modified material on the market, it has to be approved in the EU under the Directives 2001/18/EC on the deliberate release into the environment of GMOs, under the regulation (EC) No. 1829/2003 on genetically modified food and feed, and under the regulation (EC) No. 1830/2003 concerning the traceability and labelling of GMOs and the traceability of food and feed products produced from GMOs. Genetic modifications have been introduced in several plant varieties, but only seeds of some plant varieties are currently authorized.

For food products where adventitious or technically unavoidable traces of material containing, consisting of, or being produced from authorized GMOs cannot be excluded, a tolerance threshold no higher than 0.9% of the food ingredients is established below which these food products shall not be labelled according to the regulations (EC) No. 1829/2003 and 1830/2003. If the novel food products shall be subject to adequate labelling according to the former regulations, the traceability of the GMOs and the products produced from GMOs at each stage through the production and distribution chains has to be known thoroughly and transmitted in writing. The words 'genetically modified' (name of the ingredient) or 'produced from genetically modified' (name of the organisms) shall appear clearly on the labelling. Authorization of placing on the market novel foods and food ingredients containing, consisting of, or being produced from genetically modified plants is regularly revised and new acts should be consulted in the EU food safety legislation.

Directive 2000/13/EC describes in detail the declaration of the ingredients on food products, but it does not offer satisfactory information to protect persons who react with incompatibilities or allergies to certain food products. In particular protein-containing foods have a potential to cause allergies in humans. A hit-

list of major serious food allergens has been created and it includes some present in vegetable products (gluten, soy, peanut, sesame products, etc.). These products should be clearly listed in the labelling of food products and stated on the label if they are in their original or derived form (Directive 2003/89/EC). The list of ingredients that are likely to cause adverse reactions in susceptible consumers is constantly examined and the European Food Safety Authority has considered that certain products of ingredients are not likely, or not very likely, to cause adverse reactions in susceptible individuals. The Commission Directive 2005/26/EC gives a list of food ingredients (including vegetable ingredients) that are provisionally excluded from the list of ingredients that are the cause of allergies or intolerances in consumers. Although there is not a common tolerance threshold for all the allergens in food products, it is proposed to define one for each allergen and to declare it in the list of ingredients of the food product whenever the content exceeds the established threshold.

Contaminants with toxicological properties such as chloropropanols are found in acid hydrolysed vegetable protein. The EU Commission has adopted measures for setting maximum levels for certain contaminants (Regulation EC No. 1881/2006). In particular, chloropropanols are formed during the hydrochloric acid mediated hydrolysis step of the manufacturing process of vegetable products when the acid reacts with residual lipids present in the defatted meal from oilseeds and proteins from maize, wheat and rice. The maximum level of 3-monochloropropane-1,2-diol in food products has been established to be 0.05 mg/kg in the dry matter. Actions taken to reduce 3-monochloropropane-1,2-diol have an impact on organoleptic quality of acid hydrolysed VPP, and the food industry has adjusted the production process of acid hydrolysed VPP to prevent the occurrence of undesirable flavour components by alkaline treatments or by combination of acid hydrolysed VPP with fermented VPP that contain lower levels of 3-monochloropropane-1,2-diol.

15.12 References and sources of further reading

ALLEN, G. (1999) *Protein: A Comprehensive Treatise*, Stamford CT, JAI Press.

ALTSCHUL, A. M. and WILCKE, H. L. (1985) *New Protein Foods*, Orlando, FL, Academic Press Inc.

BURTON, W. G. (1969) *Potato. Encyclopaedia Britannica*. Chicago, Benton.

CREIGHTON, T. E. (1996) *Proteins: Structures and Molecular Properties*, New York, W.H. Freeman.

DAMODARAN, S. and PARAF, A. (1997) *Food Proteins and Their Applications*, New York, Marcel Dekker.

DAUSSANT, J., MOSSÉ , J. and VAUGHAN, J. (1983) *Seed Proteins*, London, Academic Press.

DENDY, D. A. V. and DROBRASZCZYCK, B. J. (2001) *Cereals and Cereal Products*, Aspen, Springer.

DERBYSHIRE, E., WRIGHT, D. J. and BOULTER, D. (1976) Legumin and vicilin, storage proteins of legume seeds. *Phytochemistry*, 15, 3–24. (doi:10.1016/S0031-9422(00)89046-9).

DICKINSON, E. and BERGENSTÅHL, B. (1997) *Food Colloids: Proteins, Lipids and Polysaccharides*, Cambridge, Royal Society of Chemistry.

DICKINSON, E. and LORIENT, D. (1995) *Food Macromolecules and Colloids*, Cambridge, Royal Society of Chemistry.

DUBE, M., SCHAFER, C., NEIDHART, S. and CARLE, R. (2007) Texturisation and modification of vegetable proteins for food applications using microbial transglutaminase. European *Food Research and Technology*, 225, 287–299. (doi: 10.1007/s00217-006-0401-2).

DUNWELL, J. M. (1998) Cupins: a new superfamily of functionally diverse proteins that include germins and plant storage proteins. *Biotechnology and Genetic Engineering Reviews*, 15, 1–32.

ENDRES, J. G. (2001) *Soy Protein Products: Characteristics, Nutritional Aspects, and Utilization*, Champaign, IL, AOCS Press.

FUKUSHIMA, D. (1991) Recent progress of soybean protein foods: chemistry, technology, and nutrition. *Food Reviews International*, 7, 323–351.

GONZÁLEZ-PÉREZ, S. and VEREIJKEN, J. M. (2007) Sunflower proteins: overview of their physicochemical, structural and functional properties. *Journal of the Science of Food and Agriculture*, 87, 2173–2191. (doi: 10.1002/jsfa.2971).

GUÉGUEN, J. (1983) Legume seed protein extraction, processing, and end product characteristics. *Qualitas Plantarum – Plant Foods for Human Nutrition*, 32, 267– 303.

GUÉGUEN, J. and POPINEAU, Y. (1998) *Plant Proteins from European Crops. Food and Non-food Applications*, Nantes, INRA Editions.

HALL, G. M. (1996) *Methods of Testing Protein Functionality*, London, Blackie Academic and Professional.

HALLING, P. J. (1981) Protein-stabilized foams and emulsions. *Critical Reviews in Food Science and Nutrition*, 15, 155–203.

JERVIS, L. and PIERPOINT, W. S. (1989) Purification technologies for plant proteins. *Journal of Biotechnology*, 11, 161–198. (doi: 10.1016/0168-1656(89)90117-X).

LEWIS, G., SCHRIRE, B., MACKINDER, B. and LOCK, M. (2005) *Legumes of the World*, Richmond, The Royal Botanic Gardens, Kew.

MARCONE, M. F. (1999) Biochemical and biophysical properties of plant storage proteins: a current understanding with emphasis on 11S seed globulins. *Food Research International*, 32, 79–92. (DOI:10.1016/S0963-9969(99)00061-7).

MILLS, E. N. C., JENKINS, J. A., ALCOCER, M. J. C. and SHEWRY, P. R. (2004) Structural, biological, and evolutionary relationships of plant food allergens sensitizing via the gastrointestinal tract. *Critical Reviews in Food Science and Nutrition*, 44 (5) 379– 407. (doi: 10.1080/10408690490489224).

MURPHY, D. J. (1994) *Designer Oil Crops: Breeding, Processing and Biotechnology*, New York, Weinheim.

OSBORNE, T. B. (1924) *The Vegetable Proteins*, London, Longmans, Green.

RIAZ, M. N. (2005) *Soy Applications in Food*, Boca Raton, FL, Taylor and Francis.

ROBBELEN, G., DOWNEY, R. K. and ASHRI, A. (1989) *Oil Crops of the World*, New York, McGraw-Hill.

SALUNKHE, D. K., CHAVAN, J. K., ADSULE, R. N. and KADAM, S. S. (1992) World *Oilseeds: Chemistry, Technology and Utilization*, New York, Van Nostrand Reinhold.

SCHWENKE, K. D. (2001) Reflections about the functional potential of legume proteins—a review. *Nahrung – Food*, 45, 377–381. (doi: 10.1002/1521-3803(20011001) 45:6<377::AID-FOOD377>3.0.CO;2-G).

SHEWRY, P. R. (2007) Improving the protein content and composition of cereal grain. *Journal of Cereal Science*, 46, 239–250. (doi: 10.1016/j.jcs.2007.06.006).

SHEWRY, P. R. and CASEY, R. (1983) *Seed Proteins*, Dordrecht, Kluwer Academic Publishers.

TALBURT, W. F. and SMITH, O. (1987) *Potato Processing*, New York, Van Nostrand.

YADA, R. Y. (2004) *Proteins in Food Processing*, Cambridge, Woodhead Publishing Limited.