Plant Proteins from Legumes

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Abstract

Legumes are part of the human edible panel since prehistory times but the remains that reached our last centuries were all from a period posterior to fire domestication. In all parts of the world where human civilizations developed, pulses were associated with cereals and the combination of their proteins managed to cover the essential amino-acid requirements of Humans and animals. Legumes gathering more than 19,000 different species, all present high protein content due to specific symbiosis with *rhizobia* and arbuscular *mycorrhizae* present in the soils. These associations are thought to originate from first symbiotic events dating from more than 60 million years before present. They allow the plants to fix nitrogen that is used for protein biosynthesis. The nutritional value of actual pulses is generally higher than that of other crops especially since domestication and the genetic selection processes operated by humans. Beside proteins with suitable amino-acid profiles, legumes also contain digestible carbohydrates and some of them also contain fat. In some cases, these fat include polyunsaturated fatty acids that increase further the nutritional value of the corresponding legumes. However, if such valuable plants managed to survive along geological periods, it is because their evolution with their environmental pressure lead them to develop anti-nutritional substances to protect themselves from their predators. Here will be discussed some of these anti-nutritional substances, the so-called tannins, phytic acid, saponins, phytoestrogens, lipoxygenase, hemagglutinin, trypsin inhibitor, as well as allergens. Because all these substances are basically useful for the crops, it is only during processing that they should be removed. Therefore, a special focus is made on traditional versus modern recipes and industrial food processing. Their respective impacts on basic nutritional components (amino-acids, fats, carbohydrates, vitamins, and minerals) as well as on the anti-nutritional factors listed above are examined. Basically, wet processing which was most frequently developed in the past, associated orf not with fermentation or germination, is also the most efficient in removing all anti-nutritional factors.

Keywords

Legumes · Prehistoric domestication · Proteins · Amino-acid profiles · Anti-nutritional factors · Tannins · Saponins · Phytic Acids · Phytoestrogens · Oligosaccharids · Hemagglutinins · Lipoxygenases · Tripsin inhibitors · Allergens

1 Introduction

Legumes have been used for thousands of years in human nutrition and constitute one of the pillar bases of human civilization. Among other early domesticated pulses are lentils (*Lens culinaris*, L. Medic.), pea (*Pisum sativum*, L.), chickpea (*Cicer arietinum*), broad bean (*Vicia Faba*, L.), common bean (*Phaseolus vulgaris*, L.),

lupine (*Lupineus albus*, L.), peanut (*Arachis hypogaea*, L.), cowpea (*Vigna unguiculata*, L. Walp.), bambara groundnut (*Vigna subterranean*, L. Verdc), or soy (*Glycine max*, L. Merr). In all continents, pulses were domesticated although the archeological traces of these events are not always easy to show. The origin of high protein content and quality in legumes is the symbiotic nitrogen fixation by rhizobia and mycorrhizae in plant root nodules. This leads to increased production of protein and injects nitrogen into agricultural systems. Nitrogen from rhizobia symbiosis also provides residual soil nitrogen to subsequent non legume crops. Therefore, legumes have been used in crop rotations for thousands of years. The proteins of legumes are not only concentrated but also exhibit interesting amino-acid profiles that make them attractive for animals and humans. Therefore, and to allow plant survival to pest and predators, legumes progressively developed an arsenal of biochemical "weapons" to limit their palatability and digestibility. Thus beside excellent theoretical nutritional characteristics, legumes developed anti-nutritional factors such as tannins, phytic acid, saponins, oligosaccharides, hemagglutinins, lipoxygenases, protease inhibitors, or certain polyphenols. These substances help preventing large consumption of plants in raw and forced humans to invent cooking processes and specific recipes including fermentation or germination. These traditional processing practices have recently been renewed thanks to modernization of processing tools and industrialization of the food preparation. These new processes can act only partially on anti-nutritional agents. This may be a cause of increasing allergy prevalence among human consumers. Nowadays, modern recording tools allow to show that in all continents and with all legumes these allergic manifestation due to pulses consumption exist and possibly rise. This review tends to go through all these aspects giving perspective to the issue of plant proteins in human future.

2 History of Legumes Cultivation and Consumption

Domestication of a crop leads to the appearance of novel traits which can be favorable to harvest and to human uses. According to Caracuta et al. [1] experiments conducted on wild modern peas, chickpeas and lentils prove that neither harvesting of wild stands or cultivation of wild legumes results in profitable yields [2–4]. Therefore the added value got from the actual crops, directly comes from domestication. Among other traits, the seed-coat should be thinner and smoother in domesticated stocks to facilitate water penetration and germination [5]. However, using this mutation as a trait of domestication, while exploring antic farming sites, remains controversial, because several cultured legumes including lentils or grass pea do not exhibit great differences with their wild relatives [6, 7]. Oppositely, increase in seed size is considered to be one of the major domestication-traits even though this increase in seed-size does not occur at the early stage of domestication but rather later as a result of crop improvements [1]. Legumes have been associated to cereals as basic foodstuff in the human history for as long as agriculture and settlement started. The remains from excavations of the Neolithic period already, shows associations of wheat varieties and barley to lentils and peas in the Near East region

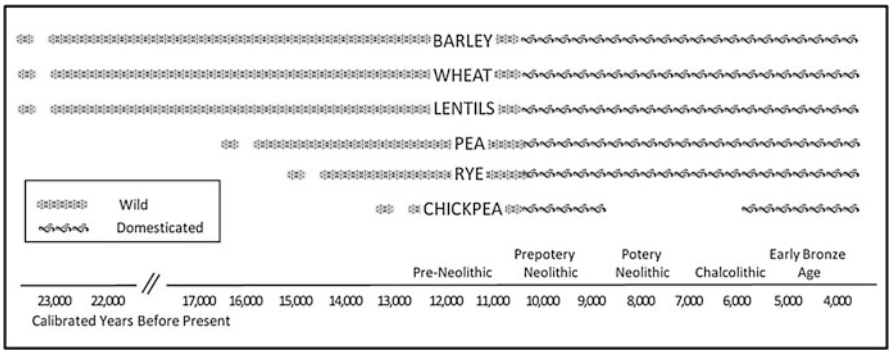

Fig. 1 Diagrammatic representation of the appearance of the main crops during human history as revealed by remains found in ancient human settlements

where cereal cultivation was shown to be first performed [8], then came chickpea (Fig. 1). The present chapter shows that all over the world and in all continents, legumes were domesticated to be part of human protein intake. They were always associated to cereals which allow the best amino-acid combination to fulfill human nutritional requirements.

2.1 Near East and Western Europe

2.1.1 Lentils

According to Zohary and Hopf [8], lentils (*Lens culinaris* L.) are found in remains which were determined to be as old as agriculture itself. Lentils seem to be closely associated with the start of wheat and barley cultivation in the Near East. The presence of small lentil-seeds was shown among remains retrieved from pre-farming zones of North Syria dated from 10,000 to 9,500 Cal BP (Calibrated Before Present). A few traces of the use of lentils are then found in Iraq, Iran, Anatolia, and Jordan. These traces are from the Neolithic period and more precisely from the ninth millennium Cal BP. and consist of small lentil-seeds (2.5–3.0 mm in diameter). This size does not allow to conclude on the wild or cultivated origin of the seeds. Larger amounts of lentil-seeds were also discovered in later phases of the Neolithic settlement in the Near East. According to Zohary and Hopf [8], they were found: in Syria (8,250 to 7,950 Cal BP) [9], in Turkey (7,800 to 7,000 Cal BP) [10], and in Iran (7,500 to 7,000 Cal BP) [11]. At that time, lentil-seeds had already attained 4.2 mm in diameter which is characteristic of a development under domestication. Lentils spread to East and then to Western Europe during the Bonze and the Iron ages, respectively. Because the remains of lentil-seeds in France seems to be less evident in early and late Bronze age than they are in Germany, Hungary, and Switzerland, it is assumed that the use of lentil-seeds spread from East to West during this particular period. However, only the seed-size of lentils can help to diagnose for domestication. Unfortunately this trait

evolves very slowly and is affected by environmental factors, and therefore, it is difficult to ascertain that we would be able to determine the one set of *Lens* domestication in the future.

2.1.2 Peas

Peas of the *Pisum* gender were found to be common in the Neolithic agriculture settlements in Europe. They were found to be closely associated with the wheat and barley products. According to Zohary and Hopf [8], remains of peas were discovered in early Neolithic farming villages of the Near East (9,000 to 8,000 Cal BP). Preserved, since they were carbonized, pea seeds were already present in aceramic jars of north Iraq, southeast Turkey, and in the prepottery B level in Jericho. According to the remains, the use of these legumes most probably spread over the Near East since remains dated from the eighth millennium Cal BP were found in this area. Remains dated from 7,400 to 7,000 Cal BP [12] show the smooth seed coat characteristic of domesticated peas. Genetic approaches show that the actual species *Pisum sativum* (L.) most probably derives from two main wild species *P. elatius* and *P. humile* which is smaller.

2.1.3 Cicer

Chickpea (*Cicer arietinum* L.) as other early European cultivated crop seems to originate from the Near East and deriving from a progenitor *C. reticulatum*. According to Garrard [13], remains of *C. arietinum* were found in Neolithic settlements of the Euphrate area and close to the natural distribution area of its putative progenitor. Later (10,000 Cal BP), it is recorded in Jericho far from its original region, and this is an indication of domestication. Establishment of the domesticated forms and replacement of the wild ancestral populations of European legumes is thought to have occurred in the Near East. This was performed within a relatively short time. Chickpea, however, appears as an exception among all other "founder crops." When all other crops including legumes are germinating in autumn, flowering in late winter/early spring and maturating in early summer, chickpea is a spring sown crop. Although the wild precursor was probably following the general cycle pattern, chickpea was deliberately sown in late winter (February) and this is considered to be a strong trait of domestication. As mentioned in [14], the winter cycle is found in all wild progenitors of the "founder crops" of Near Eastern agriculture without exception because their harvest yield is far better following winter germination. Plants are then able to use the winter rainfall for growing. It appears that chickpea sowing was delayed to avoid the *Ascochyta* disease whose pathogen agent is *Didymella rabiei*. This fungus has the potential to cause total yield loss on chickpea, and its occurrence is high since 9 fields over 10 can be attacked in a given area. In recent tests, only one out of ten actual varieties was able to resist to the fungal attacks when sowed in winter. The disease does not affect the legume sowed in late winter because of early dryness, and this allows maintaining lower (0.95 tons/ha rather than 3.0 tons/ha) but consistent yields.

2.1.4 Faba

Broad bean belongs to the section *Faba* of the genus *Vicia*. They appear today as big beans even though it is though that the progenitor of the actual broad bean was smaller and most probably derives from the *Vicia faba* var. *minor* [15]. Quite recently [1], reported the discovery of large amount of *Faba* seeds in sites located in the Galilee region of Israel. According to carbon duration, the seeds were collected 11,000–10,000 Cal BP and are the oldest *Faba* seeds ever found so far. The most ancient datation corresponds with a period when water supply was good enough for production and harvesting. The authors argue saying that these *Faba* seeds were most probably cultured under human input as early as the following millennium (10,000 Cal BP.) since seeds remains are still of good size indicating good water supply in a country where dryness was gaining. The earliest remains of broad beans found in Western Europe were from the Iberic peninsula and were dated from the late Neolithic times. In contrast, few carbonized remains of broad beans appear in several Bronze Age sites of the East Mediterranean and Aegean. All Bronze Age broad beans have relatively small seed and thus most probably belong to *Vicia faba* var. *minor*.

2.2 America

2.2.1 Phaseolus

The common bean *Phaseolus vulgaris* (L.) presents multiple variations of fresh and dried varieties. Those include string beans, green beans, French beans, kidney beans, or pinto beans. These varieties and others provide a third of daily dietary protein in some cultures of Africa and of Americas. *P. vulgaris* is originating from Mesoamerica and South America. Its domestication is much more recent than the European legumes since it is thought to start about 8,000 years before present time [16]. Plant domestication is often associated with a suite of morphological changes. In the case of common bean, domestication has led to increases in seed and leaf sizes, as well as to changes in growth habit and other features. Moreover, these morphological shifts occurred not once but twice, as common bean was domesticated independently in Mesoamerica (probably in what is now Mexico) and the Andes [17]. Currently, the domesticated gene pool of the species seems to be organized into four Mesoamerican and three Andean races [18, 19]. These two pools were domesticated independently. However, although the four Mesoamerican races Durango, Jalisco, Mesoamerica, and Guatemala differ in ecological adaptations, geographic ranges, morpho-agronomic traits, allozyme alleles, and random amplification of polymorphic DNA (RAPD) markers [18, 19], they probably all derive from a single domestication event. Indeed, they all present the same predominant phaseolin electrophoretic type S, and similar amplification fragment length polymorphism (AFLP) patterns [20, 21]. In parallel there are three Andean races: Nueva Granada, Peru, and Chile, which also differ in morpho-agronomic characters, allozymes, and phaseolin types [19]. This would support multiple domestications, but their geographic ranges overlap, and they seem to be similar in AFLP patterns [18]. This supports a single origin.

2.2.2 Lupineus

The discovery of the origin of lupine in South America is quite recent. According to Atchison et al. [22], *Lupineus mutabilis,* also called tarwi, was domesticated once and not in the putative south-central Andean core area of early agriculture, but rather in northern Peru, most likely in the Cajamarca region. This area is included, or close to, the distribution area of *L. piurensis*. Therefore, it can be assumed that *L. piurensis* is the most likely wild progenitor of the modern lupine *L. mutabilis*. Demographic analyses suggest that tarwi split from *L. piurensis* around 2,600 Cal BP. and suffered a classical domestication bottleneck. The earliest unequivocal archaeological evidence of domesticated tarwi seeds is from the Mantaro Valley, central Peru 1,800 Cal BP. According to the actual theory, lupine then spread North and South from the initial area of origin in Peru. Therefore, the pulse went south to Bolivia and north to Ecuador and Colombia. Lupine arrived then in Europe with Spanish conquistadores, *i.e.,* in the early fifteenth century.

2.2.3 Arachis

According to Bonavia [23], the geographic area of origin and domestication of the peanut would be the Huarmey valley, near the Peruvian coast. Most of the archeological records of fruits of *Arachis hypogaea* date from approximately 3,500–4,500 years BP. However, based on the distribution and biology of wild species and landraces of *Arachis,* the region of origin of the peanut may have been in the south of Bolivia and the northwest of Argentina [24]. According to Grabiele [25], radiocarbon-dated macro-botanical remains were dated from approximately Cal 7,840 year BP. They appeared as wild *Arachis* species or peanut fruits in early domestication stages. They were recovered in buried preceramic sites in the lower western slopes of the Andes in Northern Peru. However, since this region is not considered as a plant domestication center, the first *Arachis* species may have been first cultivated elsewhere in South America earlier than Cal 8,000 year BP. According to Grabiele *et al*. [25], *A. monticola* may be an intermediate tetraploid ancestor from which *A. hypogaea* has arisen upon domestication. In addition, *A monticola* was most probably obtained from the two diploid species: maternal (*A. duranensis*) and paternal (*A. ipaensis*) wild diploid species of *Arachis*.

2.3 Africa

2.3.1 Cowpea

The African *Vigna* studied here are cowpea [*V. unguiculata* (L.) Walp.] and bambara groundnut [*V. subterranea* (L.) Verdc.] [26]. *Vigna unguiculata* is most probably originating from sub-Saharan Africa. It was introduced in America during the seventeenth century by Spanish conquistadores and is now largely cultured and consumed as black eyed pea in USA and Brazil. According to Kongjaimun et al. [27], cowpea was domesticated from wild cowpea in Africa. In 2007, D'Andrea *et al*. [28] reported the discovery of cowpea remains in Kintampo settlements in Ghana. Kintampo is a Later Stone Age (LSA) tradition of West Africa dating to

3,600–3,200 Cal BP [29]. One seed collected in the B6B site in the Horizon 4 was submitted for radiocarbon dating by AMS. The resulting date was of 3,410 ± 60 Cal BP, at 95.5% c.i. (TO11883). This date confirms a Kintampo association and is consistent with other determinations obtained from the site. Although to date, the earliest remains are originating from Western Africa, a Northern or North-Eastern origin has been argued based on the absence of true ecologically wild cowpea in West Africa [30]. However, the paucity of available data from eastern African Countries has precluded a final assessment. Based on the botanical evidences, it is thought that *Vigna unguiculata* then spread in Asia. There, the cultivated cowpea or its weedy relative was subsequently selected for the vegetable crop, yardlong bean [*Vigna unguiculata* subsp. *sesquipedalis*]. The subspecies' main characteristic is the length of its pods which can range between 35 and 60 cm long. Nowadays, cowpea constitutes a major dietary source of protein for many sub-Saharan populations. When mixed with cereals, protein quality is significantly improved [31].

2.3.2 Bambara Groundnut

The main groundnut originating from Africa is Bambara *Vigna subterranean*. It is an important food legume grown widely in semi-arid Africa and closely related to cowpea (*V. unguiculata*). Bambara shares much of its area of cultivation and origins of genetic diversity with cowpea [32]. According to Philippson and Serge Bahuchet [33], the Bambara African name ⁎/jʊgʊ appeared in the Proto Bantu language probably incorporated thank to the immigration of people from Proto Benue Congo origin. This immigration is dated from a climate deterioration that scourged Sahara and Sahel and which is dated 7,100–6,900 Cal BP. Naming a plant does not mean that it was domesticated and cultured but at least recognized as a define plant probably because of human usage. Bambara groundnut appears as two botanical forms; var. *spontanea* which is most likely the wild forms and var. *subterranea* comprising the cultivated forms. *Vigna subterranean spontanea* is restricted to an area from Nigeria to Sudan, with a center of diversity around Cameroon. *Vigna subterranean subterranea* is found in many parts of the tropics and particularly in sub-Saharan Africa. Both the wild and cultivated forms bear 11 pairs of chromosomes [34]. As mentioned here, the use of this legume by human populations of West Africa probably dates from the early proto Bantu period which seems to coincide with Ceramic Late Stone Age but this still remains to be substantiated by archeobotany.

2.4 Asia

2.4.1 Glycine

The main legume in Asia is identified as soybean *Glycine max*. China is the country of origin of soybean. Deng in [35] reports that soybean was found to be present in the Neolithic site of Baligang in the Shijiahe period (Cal 4,500 BP). According to Hymowitz [36], it was domesticated and therefore cultivated in the Eastern half of North China 3,100 Cal BP. More precisely, soybean domestication probably started

in Liaoning province because the wild soybean grows everywhere and the stages of evolution are apparent [37]. *Glycine max* belongs to the subgenus *Soja*, which also contains *G. soja* and *G. gracilis*. *G. soja*, is a wild species of soybean, found in many different environments in many Asian countries [38]. According to cytological, morphological, and molecular traits, *G. soja* is most probably the ancestor of *G. max*. On the contrary, *G. gracilis* is most probably semi-wild form of *G. max*, which phenotypic traits place it as an intermediate in the speciation between *G. max* and *G. soja*. According to Willis [39], *G. max* ancestors produce tiny, hard seeds that are useless for food unless properly prepared. Therefore, the initial use of soya bean ancestors was essentially as green manure in crop culture rotations. Since then, and until 1915, Manchuria in North-Eastern China has been the heart of soybean production in China [40]. The ancient character for soybean (shu) seems to appear on Zhou dynasty bronze vessels dated around 3,020 Cal BP [36]. Confucius documents dated from 2,500 Cal BP mentioned soybean as one of the five staple grains of China. These were foxtail millet (*Setaria italica*), broomcorn millet (*Panicum miliaceum*), rice (*Oryza sativa*), wheat (*Triticum aestivum*), and legumes essentially soybeans (*Glycine max*).

3 Origin of Protein in Legumes

3.1 An Evolution Process

Legumes are particularly rich in proteins since they produce amino acid from ammonia. This NH_3 is supplied by rhizobia symbiotic organisms which produce it from aerial Nitrogen (N_2). Archeobiology established that the symbiosis between legumes (Fabaceae) and nitrogen-fixing bacteria, the so-called rhizobia, appeared Cal. 60 million years BP [41, 42]. This long association probably explains why adaptative processes gave rise to about 19,500 legume species [43]. Molecular and genetic studies suggest that rhizobia bacteria progressively associated to the more widespread and much older endo-mycorrhizal symbiosis [44]. The association between arbuscular mycoorhizal fungi and plants involve recognition factors generated by the fungus and named Myc factors [45]. As for mycorrhizal association, the plant-rhizobia interaction is most generally initiated by a mutual recognition of molecular signals released by both symbiotic partners. It is characterized by varying degrees of specificity pre-determined by the nature and profile of seed/root exudates from the legume, as well as nodulation factors from the rhizobia [46, 47]. The plant molecules involved in the recognition step are flavonoids [48, 49].

3.2 Adaptation for N_2 Fixation

Nowadays, the association between rhizobia bacteria and mycorrhizal fungi give birth to root nodules able to capture the aerial nitrogen and to inject it into the plant metabolism. Therefore, legumes receive the bulk of nitrogen fixed by rhizobia in the form of

ammonia, which is incorporated into organic form before being exported from nodules. The formation of effective root nodules with compatible soil rhizobia allow to reduce atmospheric N_2 into NH_3 for bacterial and plant use. The symbiosis requires the close association of the bacteria and the plant and the nitrogenase enzyme complex that reduces N_2 to NH_3 is oxygen labile. Rhizobia as other soil bacteria are obligate aerobes and require oxygen for respiration and metabolism. Therefore, they should combine two opposite situations under nitrogen-fixing conditions: oxygen for their own metabolic requirements and anaerobic conditions for nitrogen fixing. To achieve this paradox, the plant and the bacteria produce a micro-aerobic environment around nitrogen-fixing rhizobia in nodules. The outer cells of the nodules play as a barrier to gaseous diffusion limit the rate of oxygen into the central infected tissue. The outer cells, their bacteroids, and plant mitochondria consume oxygen as fast as it can enter the nodules. The oxygen is directly targeted to mitochondria and bacteroids via plant hemoglobins the so-called leghemoglobins. This insures a low oxygen ratio in the vicinity of the nitrogenase enzyme complex [50]. If leghemoglobin transcription is prevented (RNAi), this leads to a higher steady-state level of free oxygen locally a lower ATP/ADP ratio and a complete absence of nitrogenase activity [51].

3.3 The Nitrogen Uptake

The symbiosis between legumes and rhizobia, at its most basic level, results in the exchange of reduced carbon from the plant for reduced nitrogen from the bacteria. Thanks to its photosynthesis the plant produces sucrose which is the primary source of reduced carbon for nodule metabolism [52]. In nodules, phosphorenolpyruvate carboxylase and malate dehydrogenase activities divert carbon flux away from glycolysis to form malate. Malate is considered to be the primary source of carbon for rhizobia bacteroids. During nodule development, bacteroids are programmed to fix N_2 and not to assimilate NH_3 into an organic form. The assimilation of NH_3 is left to the plant, and during nodule development, genes such as those encoding glutamine synthase, glutamate synthase, and aspartate amino transferase are induced. These enzymes can incorporate NH_3 into amino acids for export from nodules [53]. Noteworthy, in legumes of tropical origin, like *Glycine max* and *Vigna unguiculata,* nitrogen is exported from nodules into the plant metabolism as ureides [54]. The nitrogen exportation from the bacteroids to the plant mainly occurs via ammonia rather than via any other more elaborated substance, i.e., amino acid. This transfer most probably occurs in a passive way. However, Glutamate, Asparate, or Glutathione may play a role in the active transport of nitrogenic molecules through the symbiotic membrane separating the bacteroids and the plant.

3.4 How to Explain the Efficiency of a Tripartite Symbiosis

A co-evolution process is generally put forward to explain the specificity of the association between legumes and their symbionts [55]. However, according to

Martínez-Romero [56], in some cases there may rather be a constant selection of micro-symbionts by the plant. The symbiont is selected for its large and fast capabilities for genetic change or of acquisition of symbiotic genes [57]. Rapid changes in symbiotic nucleic acid material could have enabled bacteria to adjust and adapt to the diversification burst of legumes that occurred during the earth evolution. Many studies have been conducted in order to decipher the mechanism of the tripartite association between the plant, the bacteria (rhizobium), and the fungus (mycorrhizae). According to Maillet et al. [58], mycorrhizal fungi of the genus *Glomus* and rhizobia secrete very similar lipo-chitooligosaccharide signal molecules, the so-called Nod factors in rhizobia and Myc factors lipo-chitooligosaccharides in arbuscular endo-mycorrhizal fungi. All Nod factors are lipo-chitooligosaccharides with a β-1,4-linked N-acetyl-D-glucosamine backbone of which the nonreducing sugar moiety is substituted with an acyl chain [59]. In legumes, rhizobial Nod factors are detected thanks to two different Lys-M-domain receptors to kinase. These proteins have an extracellular domain containing three Lys-M domains, a transmembrane domain, and an intracellular kinase domain. Mutations in either of the genes encoding for these receptors can block Nod factor induced responses, suggesting that these proteins operate in conjunction [60].

4 Protein Levels Compared to Other Plants

As mentioned previously, legumes are rich in proteins because they can use nitrogen fixed by their symbiotic nodules to produce endogenous proteins. Therefore when compared to other plant families, they tend to contain a higher rates of protein (Fig. 2). Legumes proteins are classified according to their solubility properties in water, salted water, or ethanol/water solutions. Albumins are soluble in water while globulins are soluble in salt water solutions, and prolamins are soluble in ethanol/water solutions.

4.1 Specific Proteins of Legumes

The most abundant proteins in grain legumes are globulins. These are classified as 2S, 7S, and 11S globulins according to their sedimentation coefficients (S) [61]. The two main storage proteins in soybean (*Glycin max*) are glycinin (11S) and β-conglycinin (7S) [62]. They can reach 41% of the total of grain weight. The glycinins are hexamers with molecular masses of 320,000 and 375,000 Da. The subunits are composed of specific acidic polypeptide chain which molecular mass is 40,000 Da linked by disulfide bonds. In lupine (*Lupinus alba*), the storage proteins are conglutin [63]. They have been classified into four groups: α, β, γ, and δ conglutins. The α–conglutin is a hexameric protein containing monomeric unit either acidic: (α) or basic: (β) subunits with molecular weight between 42,000 and 52,000 Da and between 20,000 and 22,000 Da, respectively. The α-conglutin (11S) may account for about 35–37% of the total seed storage proteins in white

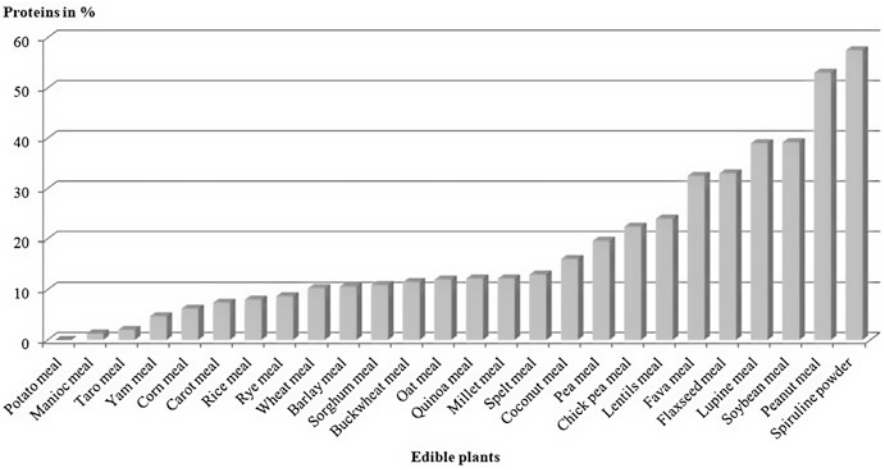

Fig. 2 Protein content of several plants used as human food

lupine seeds [64]. The most abundant lupine globulin is β-conglutin, also named vicilin, which represents about 44–45% of white lupine seed storage proteins [64]. The β-conglutin (7S) is a trimeric protein. Each of its monomer is formed by three polypeptides of low (17000–20,000 Da), medium (25,000–46,000 Da), and high (53,000–64,000 Da) molecular weight. The γ-conglutin accounts for about 5% of the amount of proteins in mature white lupine seeds [64]. The γ-conglutin (7S) is a tetrameric protein of about 50,000 Da composed of two subunits with molecular weight between 17,000 and 29,000 Da linked by disulfide bonds. Finally, δ-conglutin is the least abundant lupine conglutin, representing 3–4% only of total conglutin in white lupine [63]. δ-Conglutin (2S) is a monomeric protein consisting of two subunits of around 4,000 and 9,000 Da, respectively. These subunits are linked by two disulfide bonds. In pea (*Pisum sativum*), the globulin are named vicilin and convicilin (7S) and legumin (11S) [61]. Each group represents 4% and 3%, respectively, of the total weight of pea seeds. According to Barać et al. [65], vicilin appears as a globulin constituted from subunits with molecular weight of 47,300; 35,000; and 28,700 Da. Three minor subunits also appear in SDS-PAGE profiles of Tris-extracts with molecular weights of 37,000; 33,300; and 31,800 Da. Beside, two subunits of convicilin are identified with molecular weight of 77,900 and 72,400 Da. Legumin was identified as a trimer. Each monomer has a molecular weight of about 63,500 Da. These units associate acidic subunits of 40,890 Da and basic subunits of 22,300 and 23,100 Da. In broad bean (*Vicia faba*), the protein content accounts for 25–30% of the total weight, 75% of which are storage globulins legumin (11S) and vicilin (7S). The former is two- to threefold more abundant than the latter. Legumin is then the main storage proteins [66]. As in pea, legumin in *V. faba* is a polypeptide with a molecular weight of 60,000–61,000 Da, which is cleaved in vivo into two components: the α-subunit of 36,200 Da (acidic) and a β-subunit of 22,000 Da (basic). The native legumin molecule is composed of

six α-β units connected by disulfide bonds [66]. *V. faba* legumin in vivo is a hexamer of the basic polypeptide with a molecular weight of 328,000 Da [67]. Vicilin in *Vicia faba* has a molecular weight of 150,000 Da according to Derbyshire et al. [67], which is cleaved into subunits of molecular weight comprises between 55,500 and 33,100 Da (46,000, 43,100 and 33,300). In the common bean (*Phaseolus vulgaris*), the main globulin is called phaseolin (7S) and is a trimer formed from three subunits of 46,000; 49,000; and 50,500 Da [68, 69]. *P. vulgaris* also contains a legumin like globulin with a molecular weight of 340,000 Da. Immuno-determinations of the legume proteins show some cross-reactions between species like between peas and broad beans.

4.2 Absolute Nutritional Value

The nutritional value of different legumes can vary significantly as a result of their peculiar composition [70]. As examples, the quantity and variety of dietary fibers and starch, the protein composition, the rate of several anti-nutritional substances, and the phytochemical content of legumes can influence their dietary value. Legumes usually contain bioactive compounds, including enzyme inhibitors, hemagglutinins (lectins), phytoestrogens, oligosaccharides, saponins, and other phenolic compounds [70]. These substances can play metabolic roles in animals and humans who consume these foods frequently. The consumption of phytochemicals can be either beneficial or adverse and globally require additional investigations. They can also act synergistically or antagonistically with other components of the diet, and their mechanisms of action still have to be deciphered for health and diseases understanding. Some components are also known to improve their digestibility with food processing and this will be discussed later on. The absolute nutritional value of a legume takes into account its nutrient profiles as well as their digestibility.

4.2.1 Amino Acid Profiles

Leguminous proteins are globally low in the essential sulfur containing amino acids, methionine, cystine and cysteine, methionine, as well as in tryptophan (see Table 1) and are therefore considered to be an incomplete source of protein [80]. This is true either for humans or for domestic animal nutrition. Therefore, traditional associations are observed all around the world in human civilizations like *dhal* with rice in India, beans with corn tortillas in Mexico, tofu with rice in Asia, sorghum and cowpeas in Africa, Bambara groundnut and maize kernels in Zimbabwe, or rice and beans in Southern Africa and Latin America.

For domestic animals, the association is frequently corn with soybean or wheat and soybean [81]. For nutritional balance, legumes and cereals are to be consumed in the ratio 35:65 [82]. Although, this chapter is essentially focused on proteins from legumes, the traditional edible species also contain fibers, carbohydrates, and fat in different proportions (Table 2).

Table 1 Amino acid composition of several traditional edible legumes

	Lentils	Pea	Chickpea	Fava	Bean	Lupine	Peanut	Cowpea	Bambara	Soybean	MungBean
	L. culinaris	*P. sativum*	*C. arietinum*	*V. faba*	*P. vulgaris*	*L. mutabilis*	*A. hipogea*	*V. unguiculata*	*V. subterranea*	*G. max*	*V. mungo*
% protein	25.13	26.0	24.4–25.4	32.34	20.99	38.2	22.31**	23	18.8	38.1*	27.5
AA in % protein	Seed	Flour	Seed	Seed	Extruded flour	Flour	Nut	Hydrolysate	Nut	Flour	Seed
Asp	10.76	10.4	10.9–11.5	9.45	11.53	8.41	12.03	11.1	14.61	11.89	13.5
Tre	3.12	3.66	2.7–3.0	3.18	4.43	4.32	3.03	3.45	4.43	5.07	3.15
Ser	4.84	4.37	3.3–3.7	4.5	6.24	5.95	5.96	4.60	6.85	5.42	4.95
Glu	14.20	16.6	17.3–17.8	14.79	14.77	26.12	19.32	18.30	20.95	19.65	21.7
Pro	2.40	5.56	3.8–4.1	3.72	5.53	4.27	4.85	3.98	5.36	4.81	4.23
Gly	3.2	4.43	3.4–3.6	3.81	3.95	3.71	6.92	3.78	4.65	4.37	4.26
Ala	3.92	4.53	4.7–5.2	3.63	3.76	2.81	5.20	4.25	5.14	4.27	4.35
Cys	0.88	0.68	0.4–0.6	0.78	0.95	1.26	1.26	0.75	2.4	1.6	0.75
Val	3.98	5.2	4.1–4.6	3.81	4.81	3.52	4.21	5.98	6.24	3.4	5.20
Met	0.82	0.86	4.1–4.6	0.66	1.24	0.32	1.05	1.58	0.64	0.77	1.92
Ile	3.08	3.8	4.5–4.8	3.45	3.81	3.16	3.1	4.58	5.45	3.96	4.74
Leu	6.68	6.36	8.1–8.5	6.54	7.62	7.41	6.22	7.64	10.21	6.76	8.36
Tyr	2.52	3.05	2.6–2.8	2.91	3.57	4.28	4.27	3.16	3.13	3.53	3.27
Phe	4.36	4.54	5.0–5.3	3.75	5.72	3.25	4.09	6.60	7.69	4.33	5.66
Lys	5.72	8,58	6.7–7.0	5.55	7.10	7.59	3.58	7.28	8.02	9.08	4.19
His	2.40	3.4	2.9–3.2	2.31	3.67	3.06	2.17	3.20	3.86	3.81	2.49
Trp	0.80	0.5	0.8–0.9	0.81	1.19	0.31	0.79	ND	0.6	0.5	0.97
Arg	7.52	13.76	8.0–8.5	9.18	6.00	10.86	9.84	7.30	7.48	7.57	6.33
References	[71]	[72]	[73]	[74]	[75]	[72]	[76]	[77]	[78]	[72]	[79]

* Protein given for seed and AA for flour

** Calculated as the mean of 7 different cultivars

Table 2 Average percentages of different constituents in several crops adapted from [82]

Crop	Carbohydrates	Proteins	Fat	Fibers
Barley	83%	11%	2%	4%
Oats	74%	12%	5%	10%
Rye	82%	13%	2%	3%
Millet	75%	15%	5%	10%
Wheat	78%	14%	2%	3%
Soybean	30%	42%	21%	10%
Chickpeas	58%	25%	5%	12%
Lupines	50%	40%	7%	43%
Lentils	60%	33%	–	11%

4.3 Digestibility (AntiNutritional Factors)

All legumes presenting high protein contents together with other valuable nutrients (polyunsaturated fatty acids, minerals, fibers) have a theoretical high nutritional value. However, if such plants get through the geological times until domestication, it is because despite their nutritional value they manage to limit their destruction by potential predators, by limiting their crude digestibility and appetence. Looking closely to their composition, it can be found that all legumes contain anti-nutritional factors that limit their digestibility and therefore their nutritional interest as crude matter for animal predators in the wild. As we will see further, the consumption of these legumes raise significantly when humans were able to apply basic cooking practices. The main anti-nutritional factors being inventoried here are tannins, phytic acids, oligosaccharides, lipoxygenases, hemaglutinins, anti-protease factors.

4.3.1 Tannins

Tannins (Fig. 3) mainly contained in the seed coats [83] are defined as water-soluble polymeric phenolic compounds exhibiting molecular weights from 500 to 3000 that can combine with proteins, cellulose, gelatin, and pectin to form insoluble complexes [84].

They currently protect the grains against insects, birds, and fungal attacks. The tannins content of legumes can be rather variable between species but also inside the same variety (Table 3).

In addition, it was shown that tannins affect the availability of amino acids, the utilization of protein, and they inhibit the activities of digestive enzymes [87]. Therefore, they tend to reduce the nutritional qualities of plants for their predators [88]. In domestic animals fed sorghum rich in tannins, it was shown that there were significant inverse relationships between tannin content and the mean digestibility of all AA [89]. When fava bean hulls tannins were added to casein diet, the apparent fecal digestibility of total and individual amino acids was decreased in rats [90]. The digestibility of proline, glycine, and glutamic acid were the most affected. It was thought to be due to the interactions of tannins with the proline-rich proteins secreted by the parotid gland since increasing amount of tannin-rich fava bean hulls caused a

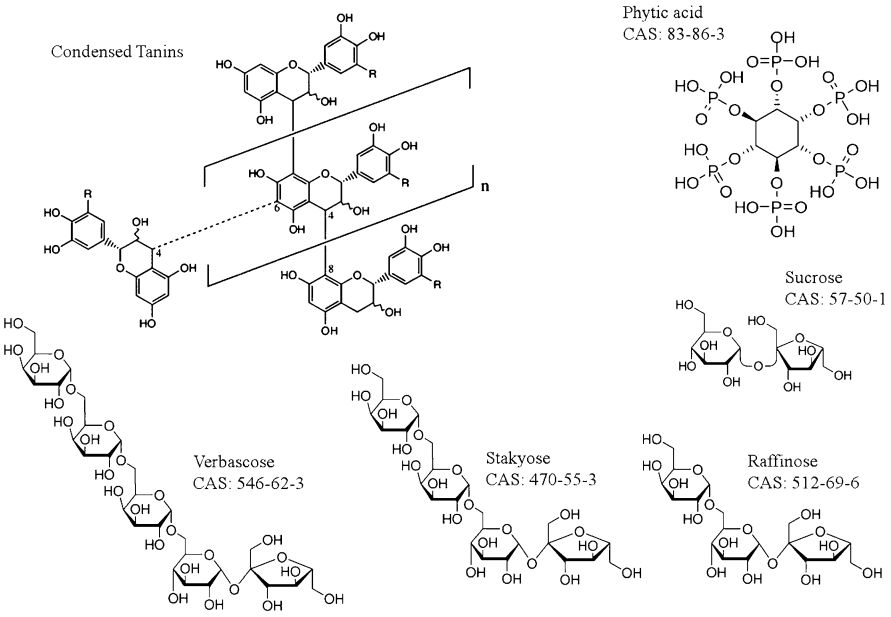

Fig. 3 Chemical structures of some anti-nutritional substances from legumes

Table 3 Tannin content in some legumes

Product	Tannin content (g/kg)	References
Chickpea (*Cicer ariterium*)	0.6–2.7	[85]
Cowpea (*Vigna sinensis*)	1.4–10.2	[85]
Pea (*Pisum sativum*)	0.6–10.5	[85]
Pigeonpea (*Cajanus cajan*)	3.8–17.1	[85]
Dry beans (*Phaseolus vulgaris*)	0.3–12.6	[85]
Kidney beans (*Phaseolus vulgaris*)	5.3–17.55	[86]
Faba bean (*Vicia faba*)	0.5–24.1	[85]
Urd bean (*Vicia mungo*)	8–12	[87]

linear increase in both the relative size of the parotid glands and in the quantity of proline-rich proteins in the rat's gland [90]. In addition, proline-rich proteins are secreted in the saliva and bind dietary tannins in the oral cavity. It was suggested that this phenomenon protects dietary and endogenous protein (digestive enzymes and proteins of the luminal side of the intestinal tract). However, if the salivary secretion is not sufficient, the tannins interactions with digestive enzymes can reduce protein and amino acid digestibility from tannin-containing diets [90, 91].

4.3.2 Phytic Acid

Cereals and legumes contain phytic acid (myoinositol 1,2,3,4,5,6-hexakisdihydrogen phosphate) at levels ranging from 0.4% to 6.4%, w/w. Phytic acid

Table 4 Phytic acid content in several legumes

Legumes	Phytic acid (g/kg)	Phytic acid (g/kg protein)	References
Soyabeans (*Glycine max*)	26	68–76	[93]
Soyabean meal (*Glycine max*)	32–41	62–78	[94]
Common bean (*Phaseolus vulgaris*)	8–11	459–578	[95]
Chick pea (*Cicer arieticum*)	5–12	29–47	[94]
Pigeon pea (*Cajanus cajan*)	7–17	29–72	[94]
Mung bean (*Vigna radiata*)	10–15	45–57	[94]
Urd bean (*Vigna mungo*)	13–15	46–54	[94]
Lentils (*Lens culinaris*)	7	27	[94]

(see Fig. 3) is the most preeminent form of phosphate storage form in most seeds. It accounts for 60–90% of total seed phosphorus [92]. In dicotyledonous seeds, such as legumes, nuts, and oilseeds, phytic acid is found associated with proteins. The amount of phytic acid in legumes can vary greatly (see Table 4).

Phytic acid has been reported to interfere with the action of pepsin on several vegetable and animal proteins *in vitro*. Its anti-proteolytic action probably goes through phytate:protein interaction that forms complexes at low pH [96]. Phytic acid was shown to inhibit trypsin activity in some but not all studies. Phytate was also shown to significantly (up to 25%) inhibit *in vitro* digestion of casein [97]. In addition, fermentation processes of millet, reducing phytate content by 23–26%, improved *in vitro* digestibility by 14–26%. Using microbial phytase, which metabolize phytate, in poultry or swine, improves phosphorous bioavailability and reduces the environmental impact of animal manure. Phytase also increased threonine and valine digestibility [98] and phytate concentration was negatively correlated with the inherent protein and amino acid digestibility of animal feed. From the animal studies, it seems that it is the ileal digestibility that is improved. The effects of phytase supplementation on protein and amino acid digestibility of proteins in several animal species may be explained by the release of protein from the protein-phytate complexes which are natural in feedstuffs. A second effect can be the prevention of formation of protein-phytate complexes directly in the gut. A third effect can be the reduction of the negative effect of phytic acid on pepsin and trypsin activities as well as to the reduction in endogenous amino acid losses [99].

4.3.3 Oligosaccharides

Legumes are classically rich in oligosaccharides of the raffinose family, namely, raffinose, stachyose, and verbascose (see Fig. 3 and Table 5).

According to Devindra *et al.* [106], raffinose family oligosaccharides are galacto-oligosaccharides that can account for more than 50% of the total soluble sugars in some cases. These carbohydrates cannot be hydrolyzed and absorbed, since the human small intestine does not exhibit the appropriate α-galactosidase activity. Therefore, these sugars are digested by the microorganisms present in the large

Table 5 Oligosaccharide content of several legumes

Genus	Species	(sample nb.)	Sucrose	Raffinose	Stachyose	Verbascose	References
Lens	Lentils		12.15	5.63	39.54	11.51	[100]
Pisum	Pea	(n = 3)	30.7 ± 1.9^{cd}	12.3 ± 2.3^{c}	31.2 ± 3.8^{a}	23.9 ± 6.7^{c}	[101]
Cicer	Chickpea	(n = 17)	18.71 ± 0.22	7.35 ± 0.05	15.35 ± 0.07	0.80 ± 0.12	[102]
Vicia	Broad bean	(n = 3)	45.1 ± 1.4^{bd}	6.6 ± 1.8^{bd}	14.2 ± 2.6^{d}	31.8 ± 5.0^{b}	[101]
Phaseolus	Kidney bean	(n = 5)	38.8 ± 5.2^{db}	7.2 ± 3.0^{ad}	48.9 ± 4.5^{e}	2.4 ± 0.5^{a}	[101]
Lupineus	White lupine	(n = 4)	24.10 ± 0.56	11.97 ± 0.41	51.87 ± 2.52	8.77 ± 1.07	[103]
Arachis	Peanuts	(n = 2)	14.22 ± 0.32	0.90 ± 0.015	1.58 ± 0.02	0.42 ± 0.005	[104]
Vigna	Cowpea	(n = 3)	29.3 ± 1.8^{c}	5.0 ± 0.1^{ad}	57.8 ± 1.7^{c}	5.6 ± 1.0^{a}	[101]
Vigna	Bambara	(n = 3)		1.71 ± 0.22	1.19 ± 0.18		[105]
Glycine	Soybean	(n = 6)	59.6 ± 3.1^{a}	7.7 ± 1.6^{a}	30.9 ± 3.9^{a}	1.6 ± 0.4^{a}	[101]
Vigna	Mung bean	(n = 4)	27.8 ± 0.7^{c}	6.1 ± 0.7^{abd}	23.3 ± 1.9^{da}	36.9 ± 1.8^{b}	[101]

Means followed by the same letter within a column are not significantly different at $P < 0.05$.

intestine. This leads to flatus formation [107] because the indigestible raffinose family sugars are fermented anaerobically by the gut flora. This causes intestinal discomfort, nausea, abdominal rumbling, and diarrhea [108]. Therefore, these oligosaccharides which are important for the plant yields can be considered as anti-nutritional agents.

4.3.4 Hemagglutinins

Hemagglutinins are proteins belonging to the lectin family. These molecules are involved in the defense mechanisms of plants via their antifungal activities [109]. They can represent a large fraction of pulse seed proteins especially in beans. These proteins are known to bind carbohydrate moieties which can be present on cell membranes. As such, they can induce cell agglutination. Phytohemagglutinins (PHA-P) are tetrameric structures associating two types of polypeptide chains called PHA-E and PHA-L. These peptides bind preferentially either to erythrocytes or to leukocytes, respectively. Thus, five possible tetrameric isolectins of approximately 120 kDa can be formed, *i.e.,* E_4, E_3L_1, E_2L_2, E_1L_3, and L_4 randomly [110]. A large channel is present in the middle of the tetramer that may protect the protein from proteolytic degradation [111, 112]. The carbohydrate-binding sites of lectins can recognize not only monosaccharides but also oligo- and polysaccharides. Both PHA subunits ("E" and "L") contain an N-glycosylation binding sites. PHA-E is specific for bisected complex-type N-glycans with an outer Gal and bisecting GlcNAc, while PHA-L specifically binds tetra- and triantennary complex-type N-glycans with β6-branching [113, 114]. Close to the carbohydrate binding sites are located the divalent cations Ca^{2+} and Mn^{2+}, which maintain an active stable conformation with a great affinity for sugars [115]. Lectins are resistant to heat and to digestive enzymes, and they also can bind to the surface of enterocytes. This phenomenon can result in toxic reactions because of changes in intestinal permeability [116]. It was shown that PHA can strongly bind to the brush border membrane of the small intestine, and undigested PHA has a dose-dependent effect on hyperblastosis, and tissue growth [117]. High doses of dietary lectins also induce the abnormal development of intestinal microvilli in rats [118] as well as the disruption of enterocytes' plasma membrane repair. This effect usually results in necrotic death of the wounded cells [119]. Blood bioavailability of lectins is poor in normal situation, but it can increase when the intestine barrier is altered.

In their study [120], Putszai and co-workers shown that lectin from *Paseolus vulgaris* were able to decrease casein digestibility in rat. The net protein utilization of crude bean was 11 and that of casein could be reduced in a dose-dependent manner when adding increasing amount of lectin extracted from *Phaseolus vulgaris*. In humans, the oral acute toxicity of *Phaseolus vulgaris* lectins is characterized by nausea, vomiting, bloating, and diarrhea [121]. The legumes' lectins are not always as toxic, and several species express moderate agglutinating effects. Finally, oral administration of certain lectins such as those from *Phaseolus vulgaris* were found to induce immunoglobulin (Ig)E-mediated reactions, and sometimes simultaneously with IgG-mediated reactions [112, 122]. These phenomena take part into the allergic reactions observed in human with several edible pulses. This will be detailed later.

4.3.5 Lipoxygenase

Lipoxygenases are non-heme, iron-containing enzymes widely distributed in plants, fungi, and animals [123]. These enzymes catalyze the dioxygenation of polyunsaturated fatty acids PUFAs into cell signaling agents used as autocrine, paracrine, or endocrine signal molecules. Multiple lipoxygenase genes were identified in plants (at least eight in soybean, *Glycine max*), in animals (at least seven in the mouse), and in humans (five pseudogene characterized) [123]. Their importance is not correlated to their concentration, and cellular effects are now known to be crucial. In plants, lipoxygenases can be found in all organs. In plants, these substances have been shown to have a role in the vegetative growth, wounding, herbivore, and pathogen attack responses and also in mobilization of storage lipids during germination [124]. The role of lipoxygenases in plant defense mechanism was shown to be due to their implications in the synthetic metabolism of signal molecules such as Jasmonic Acid and their methylated parent compounds in sorghum [125], in arabidopsis attacked by aphids [126] or in the pulse *Pisum sativum* [127]. Lipoxygenase (Lox) enzymes play a role in the development of unpleasant flavors in foods containing legumes by oxidation of polyunsaturated fatty acids. This is particularly true for soybean which contains significant levels of these substances [128]. The action of lipoxygenase is thus deleterious to the palatability of legumes rich in fatty acid such as soy or peanut and these enzymes can therefore be considered as anti-nutritional factors.

4.3.6 Saponins

Saponins constitute a class of glycosides found essentially, but not exclusively, in plants. These substances include a steroidal or triterpene aglycone linked to one, two, or three saccharide chains. The carbohydrate chains can vary in size and complexity via ester and/or ether linkages (Fig. 4).

The most common sugars linked to the sapogenin moiety are galactose, arabinose, xylose, and glucose. Saponins express amphiphilic properties thanks to the lipophilic and lipophobic characteristics of the aglycone and carbohydrate moieties, respectively. Saponins occur in numerous edible plants, including legumes (soya, peas, and beans), root crops (potato, yams, asparagus, and alliums), or medicinal herbs (ginger). In grain legumes, saponin contents vary between 0.5% and 5% dry weight, with soybean exhibiting the highest rate (5.6%) [129]. *In vitro*, saponins were shown to inhibit, carrier-mediated galactose transport but not that of L-glucose [130]. Polyethylene glycol 4000, which transfer through the endothelial barrier is known to proceed via an extracellular mechanism, is also increased *in vitro* [130]. This indicates that saponins inhibit active transport and simultaneously increase the general permeability of the enterocyte barrier. This phenomenon is observed *in vitro* at saponins concentrations ranging from 0.3 to 8 mM. Not all saponins exhibit the same potency. Therefore, it appears that some saponins are able to increase the permeability of the small intestinal mucosal cells, facilitating the uptake of substances to which the gut would normally be impermeable. This effect, occurring at rather high concentration, is likely to have *in vivo* consequences when the saponins ingestion is recurrent. These consequences may be deleterious and justify the anti-

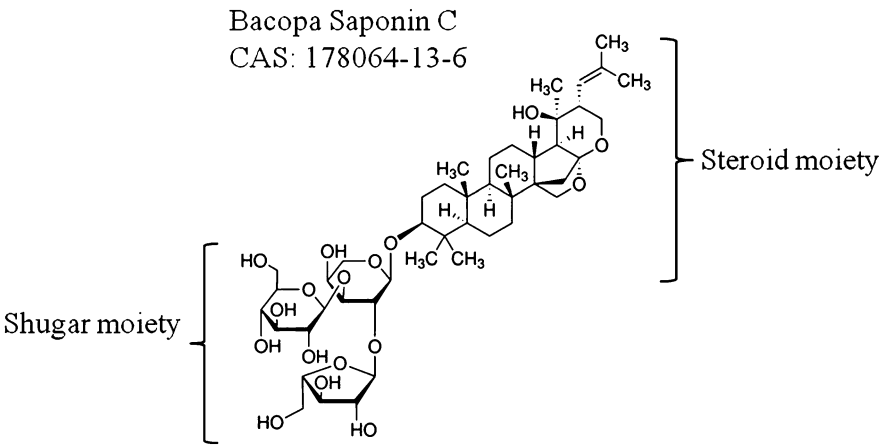

Fig. 4 An example of saponin. Here is Bacopa saponins C with its carbohydrate moiety and its steroidal moiety

nutritional status of saponins. However, saponins also exhibit anti-inflammatory, immunomodulatory and antifungal and antimicrobial activities and are protecting agents for the plants.

4.3.7 Anti-Protease Factors

Proteinase inhibitors are ubiquitously produced since their role is to regulate the proteolytic activity of their target proteinases. They are key factors in living organism interactions, and they appear essential looking at microorganisms and plants interactions, or examining microorganisms and animals interactions. They are also important in the plants and animals interactions. Therefore, many vegetables including legumes, cereals, potatoes, and tomatoes contain protease inhibitors that act on trypsin, chymotrypsin, or carboxypeptidases [92]. Maybe because of its high protein content, soybean is the richest source of dietary trypsin inhibitors and contains the Kunitz inhibitors (see Table 6) and the Bowman-Birk inhibitors [108].

The Kunitz inhibitors have a molecular weight of about 21.5 kDa with two disulfide bridges and act mainly against trypsin. The Bowman-Birk inhibitors have a molecular weight of about 8 kDa formed by 60–90 AA residues and numerous disulfide bonds. They mainly inhibit chymotrypsin and trypsin at independent binding sites. The Bowman-Birk inhibitor (BBI) family is the most widespread group in common bean (*Phaseolus vulgaris*) as well as in other legumes such as soybean (*Glycine max*) and pea (*Pisum sativum*). Variants of these two main types of inhibitors have been characterized with different amino acid sequences, electrophoretic mobility, specificity, and sensitivity to thermal inactivation. The actions of soybean inhibitors were reported to be similar in rat and humans on trypsin and chymotrypsin [129]. According to Lin *et al.* [136], Bowman-Birk inhibitors inhibit trypsin via binding with lysine or arginine at the P1 residue. Soybean, as one of the most concentrated legumes in anti-protease, has been largely studied and therefore the anti-nutritional effects of anti-

Table 6 Values of trypsin inhibitor activities in some legumes

Legumes	Trypsin inhibitor activity (mg/g)	Trypsin inhibitor activity (mg/g protein)	References
Soybean (*Glycine max*)	16.7–27.2	34.7–122.6	[131]
Pea (*Pisum sativum*)	2.7	11.9	[132]
Kidney beans (*Phaseolus vulgaris*)	4.6	13.5–62.3	[86, 133]
Chick pea (*Cicer ariterium*)	1.7–8.5	8–39.5	[134]
Cowpea (*Vigna sinensis*)	1.7	6.4	[135]

protease will be largely illustrated from data obtained on this legume. Many studies reported the deleterious effects of raw soy-protein compared to processed matters on protein digestibility and animal growth [137, 138]. Raw soybean protein preparations can cause pancreas hypertrophy and hyperplasia in susceptible animals since the exposure to soybean trypsin inhibitors results in the increased synthesis and secretion of proteases (such as trypsin, chymotrypsin, and elastase) [139]. Trypsin and chymotrypsin being rich in sulfur-containing amino acids such as methionine and cysteine, the result of this hyper-secretion is a diversion of these essential AA from the synthesis of body tissue proteins. This can result in an alteration of growth in domestic animals since soy protein is also deficient in these amino acids [140]. Direct infusion of the Bowman-Birk inhibitor purified from soybean into the duodenum of men significantly increased the pancreatic secretion of trypsin, chymotrypsin, and elastase. This effect was similar to that observed in rats [108]. Still according to Liener [108], the increased secretion of sulfur-AA-rich proteases would be due to the suppression of the negative feedback regulation of pancreatic secretion via an increased release of cholecystokinin from intestinal mucosa. This release is not only controlled by the protease secretion [141]. Because of their depressive effects on growth and protein digestibility, protease inhibitors from legumes can be considered as anti-nutritional factors. The studies earlier showed that heating and food processing can have beneficial effects on these anti-nutritional substances.

5 Food Processing

As seen above, legumes are at the same time rich in highly nutritive compounds and also in anti-nutritional factors. Their human consumption has been significantly detected during the Pre-neolithic and Neolithic periods when fire was already domesticated. Therefore, these pulses were likely to be cooked before consumption. Indeed, it can be easily demonstrated that the anti-nutritional factors detailed earlier can be partially or totally inactivated by food processing. Traditional heating, boiling, cooking, soaking, simmering, germinating, or fermenting all have been studied and were shown to improve the digestibility of the proteins in legumes. Modern practices including industrial defatting, roasting, extrusion, or

microwave cooking will be analyzed regarding their effect on anti-nutritional factors and bioactive polyphenols.

5.1 Traditional Processing

Legume seeds can be eaten raw as for chickpeas or broad beans when they are still green and at a tender stage (unripe stage) [132]. To improve conservation, legume seeds are traditionally dried to be consumed at distance of the harvest. Most farmers in developing countries naturally dry their mature beans under the sun [142]. Because seed coats contain tannins and other anti-nutritional factors, seeds are often dehulled [142]. Traditional dehulling was performed using a stone grinder and shaking the resulting grains over large flat baskets. More recently threshing, shelling, and grading of legume seeds were mechanically improved using machines [142]. After drying, seeds can be stored for several months before processing. Then two kinds of processes can be separated: the non-fermentation processes and the fermentation processes.

5.1.1 Traditional Non-fermentation Processes

These include sprouting of the fresh seeds and traditional soaking and cooking practices in boiling water. In [143], Khokhar and Chauhan studied domestic methods of processing and cooking the moth bean. Their studies included soaking in plain water, soaking in mineral salt solution, ordinary cooking of soaked seeds, sprouting, and ordinary cooking of sprouts. They described the soaking procedure as follow. After seed cleaning from broken seeds, dust, and other foreign materials, soaking was performed in tap water or mixed salt solution for 12 h at tropical temperature (24 °C). A seed to water ratio of 1:5 (w/v) was used. The unimbibed water was discarded. The soaked seeds were rinsed twice with water and then dried in air. The ordinary cooking procedure was described as follow. Presoaked seeds, after rinsing in water, were put in pans and tap water was added in a 3:1 v:w ratio. Cooking was performed for 60–90 min in water until seeds became soft when felt between fingers. Water was discarded before seeds were dried in air. Khokhar and Chauhan [143] also described the domestic sprouting procedure as follow. The soaked seeds were germinated in clean dishes lined with wet vegetal mesh for 60 h at 25 °C, with frequent watering. Sprouts were then rinsed in water and dried under the sun and cooked till soft like soaked samples mentioned above. For soybean [142], non-fermentation processes include the preparation of soy "milk," soy-cheese (*Tofu*), and soy-sheet (*Yuba*). In all cases, soybeans are cleaned and soaked in water for at least 6 h at room temperature. They can be dehulled before soaking or cooking. For soy-juice, the traditional practice usually includes a precooking step in water lasting 10–30 min. This step usually allows eliminating dust residues with the first water discard. Seeds are then crushed in the water before the final cooking step, lasting additional 10–30 min. Then the water is filtered to collect the juice. The filtered residue (Okara) can be kept for animal feeding. Tofu was traditionally prepared from the juice after cooling. Curdling agents are added to the juice. These can be calcium

chloride, calcium sulfate, magnesium chloride, magnesium sulfate, calcium acetate, or calcium lactate according to Prabhakaran *et al*. [144]. In China, calcium salts mined from mountain quarries have been used for over 2000 years [145]. In Japan, the traditional curdling agent was sea salt that contains small quantity of magnesium chloride ($MgCl_2$). The curd is then drained on a wooden frame with holes in the bottom and coated with fabric. The soybean curd is then pressed using a specific lead to squeeze out the water. This water constitutes the *tofu whey*. According to Maneepun [142], *Yuba* production is a domestic practice only performed at small scale. During thick soy "milk" prolonged cook, a film is formed at the surface. Boiling is prolonged until the sheet of film is thick enough, and then it is removed from the surface of soy "milk" by using a bamboo stick. The wet sheet is placed over a sheet of thick cloth, and air-dried by hanging. *Yuba* is used for wrapping meat and vegetable fillings in Chinese cuisine.

5.1.2 Nutritional Characteristics After Traditional Non-fermentation Processing

According to the study by El Adawi on chickpea [132], boiled chickpea seeds were not significantly different from raw peas in total protein, total amino acid, and total carbohydrate contents. Boiling significantly decreased the non-protein nitrogen, ash, and fat contents due to their diffusion into the cooking water. Crude fiber was significantly increased by boiling and or soaking associated to cooking. Water-soluble vitamins and minerals tend to leak into the water and significantly decrease in chickpeas on boiling [132]. Equally, Mubarak *et al*. [79] tested domestic processes on the anti-nutritional content of mung bean. Among them, they tested traditional cooking steps such as dehulling, soaking, boiling, and germination. They showed that the protein content was only slightly affected by soaking and boiling. All treatments significantly reduced the stachyose and raffinose content of mung bean and germination reduced it to nothing. Meanwhile, starch content was only reduced by germination. When considering the anti-nutritional factors, it appears that trypsin inhibitors and hemagglutinins were destroyed by boiling but only reduced by dehulling, soaking, or germination. Tannins and phytic acids were significantly reduced by all treatments, the most effective being germination. Polyphenols under their glycosylated form also leak into the soaking and cooking water [146] Germination of chickpea seeds resulted in a significant increase in crude protein, non-protein nitrogen, and crude fiber compared to raw seeds, while ash was not significantly affected. Germination also significantly decreased the fat and total carbohydrate contents. To be more precise, raffinose, stachyose, and verbascose were completely eliminated by germination and cooking in water for the latter. The same results were obtained on soybean sprouts [147]. According to El-Adaway [132], trypsin inhibitor activity was significantly decreased (−82%) by boiling. Sprouting was less efficient since it decreased trypsin inhibitor activity by only 34%. Hemagglutinin activity was completely destroyed by cooking and was drastically reduced (77%) by germination. This was also observed by Khalil & Mansour [148] on fava bean seeds. According to El-Adaway [132], tannins (−52%), phytic

acid (−71%), and saponins (−48%) in chickpeas were significantly reduced by simple boiling. Germination was less effective than boiling in reducing tannins and saponins but more effective in reducing phytic acid. This could be due to the phytase activity during germination. Polyphenols in legumes are generally present under their glycosylated forms and as such are soluble in water. Therefore, in all the preparation associating soaking, boiling, or simmering in water, these polyphenol concentrations can be reduced. For isoflavones, the longer the water contact the lower the remaining concentrations [149]. If the soy "milk," *Tofu,* and *Yuba* are considered, the successive soaking, cooking, and simmering in water classically included in their traditional recipes were most likely to reduce their isoflavone concentrations. This is sustained by the study by Liu et al. [150] showing that the vast majority of rural Chinese women were exposed to isoflavone levels lower than 15 mg/day when the actual exposure in modern Asian countries is usually over 20 mg/day and may go up to 120 mg/day. The estrogenic activities of isoflavones were observed at already 45 mg/day in American women [151].

5.1.3 Traditional Fermentation Processes

Legumes were fermented either traditionally or recently using several microbial strains from *Lactobacillus*, *Bacillus*, *Aspergillus*, *Rhyzopus*, *Actinomucor,* and *Saccharomyces* genders [152]. Traditionally fermented soy-food was quite numerous and still include *Natto*, *Miso*, *Tempeh,* fermented *Tofu* (*Sufu*), and soy-sauce (*Si-iu*). *Natto* is produced from soybeans cooked for 2–4 h in renewed water and dried after water discard. Beans are then wrapped in straw and seeded with *Bacillus subtillis natto*. Fermentation is prolonged for 24–48 h. Traditionally in Japan, *Miso* is made from soybean cooked in renewed water for 2 to 4 hours before grinding. Ground soy paste is then mixed with Koji (*Aspergilus oryzae*) developed on rice and/or wheat and other microbial strains together with salt and fermented in anaerobic conditions for 6–8 month. *Miso* tends to resemble the Chinese soy paste (*Tao-cheow*) [142]. *Tao-cheow* is made by incubating soybean paste with *Aspergillus oryzae* or other microbial strain like *Lactobacillus delbrulckii*, *Pediococcus halophilus*, *Saccharomyces* sp. for 3–4 months. After this first stage of *Koji* fermentation, a liquid is formed and separated for production of soy sauce. The resulting paste is packed and pasteurized and can be kept for several years with the best flavor after a year. *Tempeh* is an Indonesian soy-food prepared from soybeans rinsed and precooked 2–3 times in renewed water before being light dried and seeded with *Rhyzopus oligosporus*. Traditionally, precooked seed were packed in banana leaves and the fermentation was performed for 24–48 h at tropical temperature 24–26 °C, with high hygrometry [149]. *Si-iu* production as described in [142] includes two fermentation steps. The first stage is koji production using *Aspergillus orizae* in an aerobic solid-state fermentation for 3–4 months. The second stage is aromi fermentation by mixed cultures of halophilic yeast and lactic acid bacteria. This is an aerobic fermentation in 20–22 percent (w/v) brine solution leading to a bright reddish-brown colored sauce with pleasant aroma and salty taste [142].

5.1.4 Nutritional Characteristics After Traditional Fermentation Processing

Fermentation usually induces phytate hydrolysis because microorganisms possess phytase enzymes, which hydrolyze phytic acid into inositol phosphates [153]. This phenomenon is valuable because myoinositol phosphates with less than five phosphate groups (*i.e.*, IP-1–IP-4) do not inhibit zinc absorption [154], and those with less than three phosphate groups do not affect nonheme iron absorption [155, 156]. Microbial phytases originate either from the microflora present on the surface of legumes or from inoculates used for processing. The phytate reduction can vary and can reach −90% in soya beans, cowpeas, and lima beans. When tannins content are high, the phytase activity can be inhibited, and fermentation is less-effective. Fermentation also improves protein quality and digestibility as well as vitamin B content. Fermentation reduces adverse microbial development and improves food safety. Fermentation also produces small organic acids that improve iron and zinc absorption. This leads to lower pH and increases the activity of endogenous phytase from legume flours [157]. Ibrahim *et al*. [158] also showed in cowpea that fermentation with *Rhizopus oligosporus* dramatically reduced trypsin inhibitor activities. It was the same when fermentation was performed using a lactic acid bacteria *Lactobacillus plantarum* DSM 20205. Note that again in all recipes soya beans were traditionally cooked for long durations (2–4 h) in water and that the water was discarded. Therefore, glycosylated isoflavones were most likely eliminated with the water revealing that the isoflavone exposure probably raised dramatically in recent times in soy consuming countries where industrial processing were developed. Indeed, in industrial Asian countries, the modernization of human soy-food processing occurred 50 years ago, *i.e.*, about two human generations ago.

5.2 Modern Processing

Soy being widely used in industrial countries, either for animal or human feeding, is probably the legume which has been the most intensively processed to adapt to different people tasting and uses. New processes are still extensively developed to fit new demands [145]. As an example, food companies developed soy cakes, soy flakes, soy protein concentrates or isolates, meat substitutes proteins and extenders, and improved the quality of final products. Modern processes include high pressure cooking, autoclave cooking, microwave cooking, and extrusion. Solvent extractions are also modern processing.

5.2.1 Non-Fermented Modern Legume Products

According to Noguchi [145], modern *Tofu* is made from grade soybeans soaked overnight in water. After water discard, boiling water is poured on the beans which are pulverized into a mash. The paste is then ladled into boiling water and allowed to boil gently for about 10 min. Then the mixture is filtered to obtain “soy milk” and the residual material is called *Okara*. In Western countries, the process can be even simpler since after boiling for 1–2 min, softened soya beans are ground in cooking

water before being filtered to collect "soy milk" and *Okara*. In Japan, modern cooking of the beans were developed consisting in quick steaming at high temperature (45 seconds at 180 °C) before crushing and water addition. Subsequent filtration leads to the separation of "soy milk" and *Okara*. A small amount of either calcium sulfate ($CaSO_4$) or magnesium chloride ($MgCl_2$) from gypsum is added to coagulate "soy milk." The curds are then gently removed from the top of the whey and poured into molds lined with cloth. The containers have many draining holes in their bottom to evacuate whey. Other derivatives from "soy milk" or *Tofu* are *Yuba* (soymilk sheets) and *Shimi-tofu*. *Yuba* is made heating thick "soy milk" in pans to evaporate the water. The thin film formed on the surface of "soy milk" is then gently removed and dried. *Shimi-tofu* is prepared from soy protein curd cooled to below 0 °C. During this step, small ice crystals grow, before being thawed to expel excess water. A dried matter is then formed having a sponge-like texture named *Shimi-tofu*.

Beside these modern making of traditional products, new technologies allow producing new types of products based on legumes. The Fig. 5 summarizes the different ways followed to obtain different modern products from soy.

Soy cakes are essentially used for animal feeding. They are obtained from soy oil processing, after the oil extraction by pressing and/or using solvents like hexane. For human feeding, soy meal and oil are the most frequent soy-product, from which most other processed soy-based products derive [142]. New technologies have been introduced in the soybean industry that have and will have significant impact on farming methods, storage, and distribution of legume-based products. The industrial

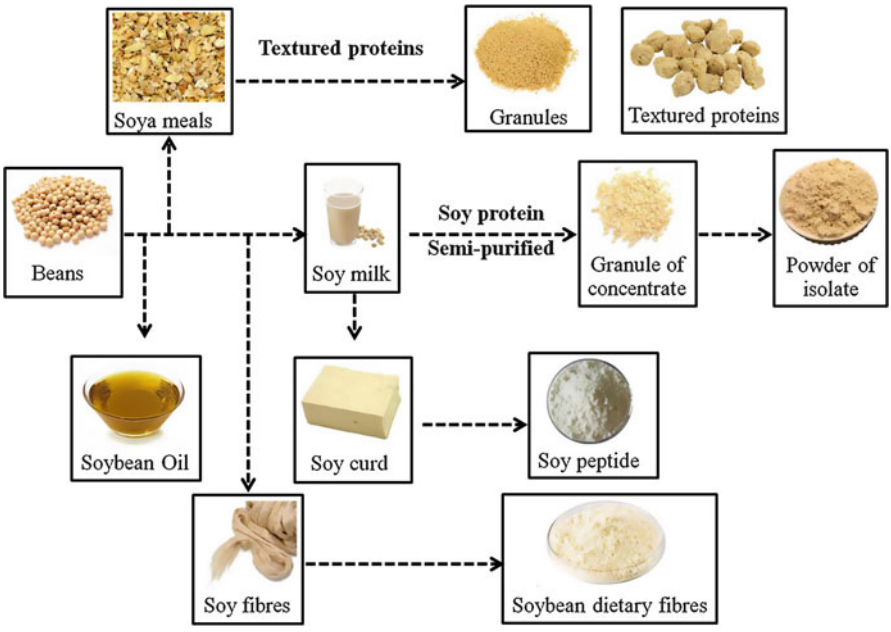

Fig. 5 Different soy-based products and their pathway of production following modern processing

processes always start with cleaning the seeds to remove foreign material. After drying and cracking into pieces, seeds are dehulled. They are heated and rolled into "flakes" before oil extraction. Oil is extracted from soybean flakes using hexane and then degummed and refined for edible and non-edible uses. The protein-rich flakes are toasted, dried, and ground in meal. Soybean meal can be mixed with corn for use in animal feeding. It is also processed into soy flour, soy concentrates, or soy protein isolates. The degumming process of soy oil gives lecithin, which is used in the candy and baking industries. Full fat soy flour from soybean is also used for baking, for soy-based beverages, snack foods, as well as for traditional foods (soy sauce, *Tofu*, and *Miso*). Texturized vegetable protein (TVP) obtained by extrusion of defatted soy flour can be found in different forms (granules, flakes, chunks, or slices). TVP has a long shelf life and can be kept at room temperature for several months. TVP should be re-hydrated with equal quantity of water and can be used as a meat substitute in processed foods (baked goods, meat products, protein drinks, soup bases, and gravies). Microwave and autoclave cooking also are modern processes which are used to prepare canned legumes.

5.2.2 Fermented Modern Legume Products

According to Nogushi [145], modern *Miso* is no more prepared from soya bean boiled for several hours in renewed water but rather from steamed beans. The process still implies long-term fermentation with *Koji* from soya, rice, or wheat and with other microorganisms. Although soy sauce still requires time to be brewed as it is for fine wines and cheeses, modern soy sauce is made from roasted wheat grains crushed and soybeans softened by steaming. A special seed starter is then added to the wheat and soybean mixture and incubated for 3 days. The resulting *Koji* is then combined with brine to form *Moromi*. *Moromi* is then fermented in large tanks until it reaches its full flavor and then pressed into fabrics to extract the raw soy sauce. The latter is then refined and pasteurized before its packaging in bottles.

5.2.3 Nutritional Quality of Legume Under Modern Processing

According to Siulapwa and Mwambungu [159], after oil solvent extraction, soybean seeds moisture is higher than that of row beans and that of extruded full-fat soy seeds. These have lower ash content when compared to raw seeds or to seed extracted with solvent. The extruded product exhibit lower fat and higher protein content than raw seeds and seeds extracted with solvent. Extrusion and solvent extraction reduce crude fibers, Ca^{++}, and phosphorous content. Both processes reduce significantly trypsin inhibitors activities. The processes increase arginine proportion while they decrease methionine. Raw seeds had lower amount of all the nonessential amino acids apart from tyrosine when compared to solvent extracted seeds and extruded seeds. In cowpea (*Vigna unguiculata*), Ibrahim and coworkers [158] showed that long-time soaking (16 h) in bicarbonate solution caused remarkable reduction in the anti-nutritional factors. Pressure cooking was more effective than ordinary cooking to remove phytates. This was confirmed by Khokhar *et al.* [143] on moth bean (*Vigna aconitifolia*) cooked under pressure after soaking in mineral salt solution. Finally, El Adawi [132] showed on chickpea (*Cicer arietinum*)

that microwave cooking reduced non-protein nitrogen, ashes, fibers, and oligosaccharides but had no significant effect on total protein and carbohydrate content including starch. In their study, they also show that this process also significantly reduced trypsin inhibitors and hemagglutinin activities as well as tannins, saponins, and phytic acid. Chickpea autoclaving led to the same reduction of anti-nutritional compounds except for trypsin inhibitors for which it was shown to be more efficient. However, both cooking processes significantly depressed the B vitamins content (Riboflavin, Thiamin, Niacin, and Pyridoxine) sometimes at rates over 50%. Minerals including Na, K, Ca, Mg, P, Mn, Zn, Cu, and Fe tend to leak into the water while boiling. However both microwave and autoclave cooking tend to better preserve their concentrations compared to other practices. These results were remarkably confirmed by Mubarak [79] while working on mung bean seeds (*Phaseolus aureus*). The effect of fermentation was studied in beans (*Phaseolus vulgaris*) [160]. The parameters followed were anti-nutritional factors (α-amylase inhibitor, chymotrypsin inhibitor, cyanogenetic glycosides and lectins) and also fibers (total dietary fiber -TDF-, insoluble -IDF- and soluble -SDF-). Beans were treated by natural and lactic acid fermentation. Autoclaving was added or not after the fermentation process. All treatments decreased the SDF content, while the IDF content were not modified in processed beans. Cellulose content was reduced by treatments and resistant starch increased in processed beans, except after lactic acid fermentation. Fermentation with *Lactobacillus plantarum* increased pectic polysaccharides and Klason lignin. Microorganisms reduced the solubility of dietary fibers. According to Noguchi [145], cooking soybean mash for *Tofu* and "soy milk" in water for at least 10 min before filtering "soy milk" is crucial since anti-nutritional enzymes of the beans are inactivated during boiling. This is confirmed by Chen and co-workers [161] who showed a progressive decrease of trypsin inhibitor activities (Kunitz and Bowman Birk factors) in "soy milk" with or without added salt. In their experiments, 10 min cooking approximately decreased TI activities between 40% and 30% of their initial value. One hour cooking reduces it between 20% and 15% of the initial value. However, not all modern processing of "soy milk" and *Tofu* especially those developed in the West include such step. Consequently, it has been shown in 1997 in Japan [162] that soybean products retain 2.5–12.5% of the initial trypsin inhibitor activity of the whole soybean and that "soy milk" is the food which is the most concentrated. Thus humans are consuming some active trypsin inhibitor in their daily lives. In [163], the phenolic composition of mung beans (*Vigna radiata*) and yellow soybeans (*Glycine max*) were followed under soaking and fermentation using *Lactobacillus plantarum* CECT 748 T. It was shown that soaking induced leaking of conjugated isoflavone for soybeans and increased apigenin derivatives in mung beans. On the other hand, fermentation converted glycosylated isoflavones of soybean into bioactive aglycones and increased the bioactive vitexin in green beans. These data are, in accordance with those from Fernandez-Lopez *et al.* [149], showing that glycosylated isoflavones tend to be progressively removed from soybean matrices during prolonged cooking and simmering in water. These steps were included in soybean traditional recipes. However, nowadays, modern processes tend to replace boiling in water by steaming and

cooking times and contacts of seeds with water were dramatically reduced in modern practices. In addition, extruded soy still contain high amount of isoflavones although they tend to be deglycosylated during the extrusion process [164]. This means that isoflavone exposure of soy-consumers was considerably lower in ancient times than it is nowadays when people consume modern processed soy-products. Because of their estrogenic effects, isoflavones tend to reduce the fertility of the soy consumers (animals and most probably humans) and can therefore be considered as anti-nutritional factors. In addition, the modern environment contains many other endocrine disruptors of anthropoid origin which can act synergistically with isoflavones on reproduction or estrogen-dependent tumor growth [165]. Isoflavone can also exert beneficial effects on several health end-points (bone preservation, prostate cancer protection, breast cancer occurrence, hot flashes relief...) and should be kept for specific health application for specific consumers [149, 166].

6 Allergy

Food allergies are adverse reactions to a harmless food that occur when the immune system reacts to proteins normally present in food without any incidence and that are recognized as foreign substances in some individuals. Then, the immune system triggers a response to neutralize them. Allergic responses vary from person to person and a protein may be allergenic in one individual but not in others. Nowadays, according to Verma *et al*. [167], eight foods or food groups account for over 90% of food allergies (peanuts, soybeans, cow's milk, hen's egg, fish, crustacean, wheat, and tree nuts). According to Maria John *et al*. [168], soybean is listed among the eight first allergenic diets for humans and animals due to the presence of many kinds of allergens whose action can be amplified by the presence of other anti-nutritional factors. Overall, allergenic events recorded after consumption of legumes in decreasing order of frequency may be peanut (*Arachis hypogaea*), soybean (*Glycine max*), lentil (*Lens culinaris*), chickpea (*Cicer arietinum*), pea (*Pisum sativum*), mung bean (*Vigna radiata*), pigeon pea (*Cajanus cajan*), and lupine (*Lupineus alba*). This takes into account both the allergenic power and the overall consumption at world scale. In Spain in 2015, [169] reported that among 455 adults, the prevalence of legumes allergy was 6.9% (77% women). Legumes involved were: lentil 27% of episodes; bean, 19%; peanut, 16%; soybean, 14%; chickpea, 13%; pea, 8%; and mungo bean, 3%. Anaphylaxis due to food allergies has a prevalence of about 6–8% in children and 4% in adults [167].

6.1 The Proteins of Food Allergies

Food reactions can be classified into three groups: the non-immunologic reactions and the immunologic reactions that can be subdivided again according to the type of reaction involved. The non-immunologic reactions include food intolerance or gluten sensitivity whereas immunologic reactions can either induce immunoglobulin

E (IgE)-mediated symptoms or non-IgE-mediated gastrointestinal symptoms. These phenomena mostly occur early in life, and food allergy frequency usually decreases with age. Legumes usually provoke IgE-mediated reactions. These reactions occur because foreign antigens have been able to stimulate the antigen-presenting cells at the gut level. This means that proteins were able to resist to food processing and then to gastric degradation by local acid and protease. The antigen presenting cells then process the antigens and present them to the Major Histocompatibility Complex (MHC). This activates naive CD_4^+ lymphocytes of the Th_2-type.

These immune cells secrete IL-4 and IL-13, which promote specific IgE secretion by plasma cells. This leads to the IgE antibodies to bind to mast cells. Re-exposure to the same food antigens causes the antigen-IgE antibodies complex to bind to mast cells. This directly provokes mast cells degranulation and release of histamine. Histamine and other mediators (prostaglandin D2, and cysteinyl leucotriene) can provoke vasodilatation, smooth muscle contraction, and mucus secretion that all lead to development of allergenic symptoms [170]. As mentioned earlier, allergenic proteins share common physicochemical and immunological properties. Class 1 food allergens such as legume allergens are stable in gastric fluid; their molecular mass range between 10 and 70 kDa; they are water soluble glycoproteins and can lead to sensitization via gastrointestinal tract [171]. Class 2 food allergens are sensitive to heat and to gastric acid and enzymes, thus sensitization does not occur via oral route. By means of IgE cross reactivity, they can cause allergenic response in people sensitized via inhalant allergens like pollen [172]. The dose of exposure does not seem to be related to either the sensitization process or to the allergenic susceptibility. Less than one microgram levels can induce an allergenic reaction [167] even if some authors report that the minimal level of exposure is over 500 μg of protein but not below this [173]. Glycosylation contributes in the allergenicity of a protein and it seems that the glycan moiety can also interact with IgE antibodies. The structural features of the proteins which are responsible for their allergenic properties are still difficult to predict. In the case of legumes, during digestion, allergens lose conformational form and exist as linear epitopes and sensitized individuals via gastrointestinal tract [67]. To summarize, the legume allergens resist to gastric fluid and proteases, they are heat stable and are glycosylated proteins. They present epitopes that bind to IgE. These can be linear epitopes remaining after food processing and gastric attack, they induce biologically active reactions.

6.2 Main Allergens from Legumes

Several classes of proteins have been involved into legume allergy. These are storage proteins which are considered to be stable to heat and to gut enzymes. Associated to anti-nutritional compounds such as saponins or hemagglutinins, they can cross the gut barrier and can induce immune response. Storage proteins presented earlier in this review are primarily localized in the seed, nut, or kernel. They are classified in Cupins, Prolamins, Pathogenesis Related proteins (PR-proteins), and Profilins.

6.2.1 Cupins Superfamily

The cupins superfamily share two conserved consensus sequence and a β-barrel core domain. Cupins include allergenic seed storage proteins of the vicilin and legumin family. These storage proteins are present in several legumes known for their allergenic properties, *i.e*., soybeans (Gly m5, Gly m6), peanuts (Ara h1, Ara h3), lupine (Lup a), pea (Pis s1), and broad bean (Vign r2, Vign r3).

6.2.2 Prolamin Superfamily

The prolamin superfamily is widely distributed among plants and not only in legumes. Prolamins include cereal seed storage proteins, several important types of allergens from legumes, tree nuts, cereals, fruits, and vegetables. This family includes chickpea allergens (Cic a2S Albumin), which is a 2S protein. It also includes the nonspecific lipid transfer proteins, the cereal alpha-amylase, and some protease inhibitors.

6.2.3 Pathogenesis Related Proteins (PR-Proteins)

PR-proteins stand for more than 10 different types of protein which increase in plants in response to environmental stresses or pathogens. PR-proteins are generally small in size, stable in acidic conditions, and resistant to proteolytic degradation. In legumes, some allergenic proteins were shown to belong to this category such as Vig r1 from mung bean, Ara h8 from peanut, and Gly m4 from soybean.

6.2.4 Profilins

Profilins are small proteins from 12 to 15 KDa. They are found in the cytoplasm of eukaryote cells and therefore they exhibit highly conserved sequences. Plant profilins are involved in a large proportion of cross-reactions between allergenic sources and especially in cross-reactions with pollens. As Class 2 allergens, they are considered to affect 10%–35% people in Europe. Some identified profilin-related legume allergens are Ara h5 from peanut and Gly m3 from soybean.

6.3 The Identified Allergens

In [167], Verma et al. gave a comprehensive table with the allergens from legumes which were known in 2012. In addition, main legumes are classified according to their decreasing importance of allergenic effects (frequency and severity) together with the number of known allergens in Fig. 6. In 2016, Bouakkadia et al. [174] completed the list that was increased to 15 allergens in peanuts, to 14 substances in soybean, and to 4 different compounds in lentils.

6.4 Effect of Food Processes on Allergen Proteins

Verma *et al*. in [175] published a comprehensive review and table showing the impact of different food processes on the inhibition of legume proteins playing as

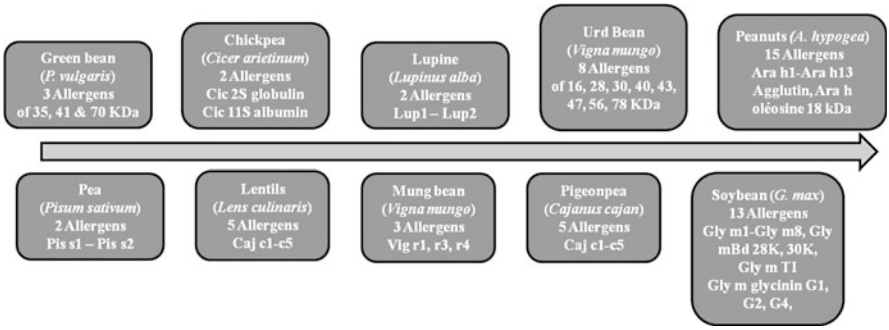

Fig. 6 Classification of the main legumes according to their increasing allergenic properties. The known allergens are mentioned for each pulse

food allergens. They classified the processes into four major categories: roasting, boiling, autoclaving, microwave heating. They reviewed the main allergens from peanuts (*Arachis hypogea*), soybean (*Glycine max*), lentils (*Lens culinaris*), lupine (*Lupinus alba*), pea (*Pisum sativum*), French bean (*Phaseolus vulgaris*), chickpea (*Cicer arietinum*), and mung bean (*Vigna mungo*). The effect of cooking has to be tested since it is not easy to predict. In some cases, cooking practices lead to elimination of the epitopes and to a reduction of the IgE reactivity. In that case, food processing affects the structural and allergenic properties of allergens by altering their stability or other physicochemical properties. On the opposite, in other cases, there is an increase of immunoreactivity of the antigens which leads to a higher IgE reactivity, a greater release of histamine and other mediators to finally increase the allergenic response. This can be explained since three-dimensional structures of proteins are generally correlated with their activity. Different temperature treatment can induce variable changes in protein structure. As an example, when heated at 70–80 °C, proteins lose their secondary structure whereas at 80–90 °C, new bonds and rearrangements of disulfide bonds can occur. At higher temperature (90–100 °C), protein aggregates can be formed [176]. Over 100 °C, new bonds between lysine residues and other substances may be created leading to the formation of adducts [177]. Protein digestibility and absorption usually increases with heat treatments. However, in some cases, thermal processing may also lead to formation of neoantigens that were not originally present and that can enhance the allergenic response. These neoantigens can result from the Maillard reaction, of protein with sugar residues upon heating. From the review of Verma *et al*. [175], it appears that boiling and autoclaving are overall good ways to reduce or to eliminate allergens although these procedures were not always efficient especially during short treatments on lentils, lupine, or French beans allergens. It should be noted that the traditional recipes which included prolonged cooking or simmering in water were empirically designed to get rid of anti-nutritional factors but can also be considered as efficient on allergens. Finally it can be summarized the following elements: Microwave heating is efficient in reducing soybean allergens but not lupine allergens. Autoclaving was reported to decrease the allergenicity of lentils, pea, chickpea,

lupine, and peanuts. Boiling is efficient in reducing the immune reactions of peanuts, mung bean, chickpea, and soybean antigens but the duration of the treatment is crucial and generally as to be prolonged for better efficiency. Roasting decreases mung bean allergenicity but increases that of peanut. As a consequence, food processing should be taken into account while comparing the prevalence of allergic reactions to various legumes over different world regions.

7 Conclusion

Proteins from legumes are undoubtedly of remarkable nutritional interest although they are better combined with cereal proteins to cover the animal food requirements. These combinations are known to exist since the domestication of crops anywhere human civilizations developed. Therefore, considering the actual expansion of the human population, pulses can be considered as a solution for future sustainable nutrition. Their ability to fix and enrich the soils in nitrogen as well as their capacity to produce protective and signal molecules to prevent or face pest attacks give them advantages when phytochemical treatments are due to be reduced. However, if these invaluable plants reached our modern times, it is because they also manage to protect themselves from their natural predators. Therefore, co-evolution of plants with microorganisms, insects, and herbivores along the geological times conducted to the intrinsic production of many different defensive substances some of which can be considered as anti-nutritional factors. These factors being essential for the plant growth and survival, genetic improvement should respect them for better field production rates. Therefore, the removal of these factors, which were developed by the plants to be detrimental for their consumers, can only be achieved at the transformation level. Future food processing methods will have to be imagined to reduce these anti-nutritional factors as economically as possible for both environment and market. Lessons should be taken from the ancient times and from the traditional practices and recipes used as early as the domestication of legumes started. Crossing different data obtained from modern practices or from those developed in the past, it appears that the traditional food processes, being essentially wet, managed empirically or not to eliminate most of the deleterious compounds including phytoestrogens, anti-nutritional substances, and allergens.

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