Review

Grain legume proteins and nutraceutical properties

Marcello Duranti *

Department of AgriFood Molecular Sciences, Università degli Studi di Milano, Italy

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Abstract

Grain legumes are a valuable source of food proteins. Their exploitation is expected to grow in relation of a growing world’s food needs. Moreover, it is currently taking place a reappraisal of the beneficial effects of legume seed dietary intake, which are the basis for various health claims. Proteins and peptides concur to the observed biological activities of legume seeds, but their effect(s) has(ve) not completely been disclosed. Aims of this review are: to report the most relevant putative positive effects of grain legumes on human health and to give an account of the current knowledge on the demonstrated legume seed protein biological activities. Specific effects on the prevention and treatment of various diseases, mostly of which are typical of the affluent countries, are reported. Examples of studies at molecular level aimed at elucidating of the underlying mechanism(s) are given. The prospects on targeted legume protein exploitation in the nutraceutical area, including the biotechnological approaches, are also considered. © 2005 Elsevier B.V. All rights reserved.

Keywords: Leguminous seeds; Pulses; Proteins; Biological activity; Functional foods

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* Tel.: +39 02 503 16817; fax: +39 02 503 16801.
E-mail address: marcello.duranti@unimi.it.

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1. Introduction

Grain legumes are important sources of food proteins. In many regions of the world, legume seeds are the unique protein supply in the diet. Very often they represent a necessary supplement to other protein sources [1]. Therefore, the dietary importance of legume seeds is expected to grow in the years for the protein (and other nutrients) demand of the increasing world population and the need of reducing the risks related to consumption of animal food sources, specially in the developed countries [2].

Recently, it is being acknowledged that food proteins are not only a source of constructive and energetic compounds as the amino acids, but also they may play a bio-active roles by themselves and/or can be the precursors of biologically active peptides with various physiological functions. From this point of view, the best known examples are casein-derived peptides which have been proved to possess immuno-modulating, anti-hypertensive, anti-thrombotic and opioid activities [3].

A new research and productive area based on these findings, which was named nutraceutics, has developed. The term nutraceutics was coined in 1979, as the result of the fusion of nutrients and pharmaceutics, by Stephen De Felice founder and President of the Foundation for Innovation in Medicine (FIM, Cranford, NJ). Nutraceutical is “any food, or part of food, considered to provide health benefits, including the prevention and treatment of disease”. Other related definitions, nevertheless conceptually different, were applied to dietary supplements (a substance produced by isolation or microbial culture purification that gives health benefits), functional food (a food engineered or supplemented to give improved nutritional value) and medical food (a food having inherent or added medicinal properties) [4]. The use of these terms is often confused and sometimes misleading, because the attribution of specific functionalities to a food or a food component is not always scientifically demonstrated since the cause/effect relationships of single food components are difficult to prove, specially in man. Nevertheless, this recent trend is contributing to add deepest meanings in the awareness that food and health go parallel. An empiric and general approach to the beneficial effects of diet on human well being consist in recommending wholesome and healthy food as the basis for disease prevention. An example of that is the American Hearth Association (AHA) dietary indication in which is strongly endorsed to follow a diet that contains a variety of foods from all the food categories and emphasizes fruits and vegetables, fat-free and low-fat dairy products, cereal and grain products, legumes and nuts, fish, poultry, and lean meats. These guidelines are only partly based on the effects of known food components, but above all they emphasize the overall eating pattern and such an approach is consistent with a wide variety of eating patterns and lifestyles [5].

As it can be seen, the above guidelines support the food use of grain legumes, i.e., the leguminous seeds which are grown for seed production and consumption. More recently, other scientists’ boards have claimed that “grain legumes effectively contribute to a balanced diet and can prevent widely diffused diseases, including type II diabetes and cardiovascular diseases” [6].

Although grain legumes are eaten by man in any country since thousands of years, only recently – say 20–30 years ago – their use as a food and their effects on man have begun to be investigated with suitable approaches. This trend is leading to a wide consensus amongst physicians, nutritionists, dieticians and most people dealing with food and health on the beneficial effects of a limited but regular food intake of legume seeds. However, which are the nutritional, pharmacological and epidemiological evidences for these claims? Which molecules are responsible for the observed effects? An exhaustive answer to these questions should be too extensive for a single review, due to the several seed components and the many facets of this topic. This review will therefore exclusively focus on the demonstrated effects of a single class of legume components, that is the protein/peptide family. As a matter of facts, this class of macromolecules is currently receiving special attention, because of the specific and direct biological activities that some of them may effect in the human body.
2. Grain legumes for food

In terms of economic importance, the Leguminosae are the most important family in the Dicotyledonae [7]. The Leguminosae is one of the largest families of the flowering plants with 18,000 species classified into around 650 genera [8]. This is just under a twelfth of all known flowering plants. The Leguminosae constitute one of humanity’s most important groups of plants. Legumes are second only to the grasses (cereals) in providing food crops for world agriculture. In comparison to cereal grains, the seeds of legumes are rich in high quality protein, providing man with a highly nutritious food resource. The major staple foods, such as beans, soybean, lentils, peas and chickpeas, are all legumes. The total world value for leguminous crops is thought to be approximately two billion US dollars per annum. Many more legumes are local food plants. In addition to those legumes mainly cultivated for human consumption, many yield important fodders, green manures and forages, e.g., *Lupinus* (lupin), *Medicago* (alfalfa) and *Trifolium* (clover). Legumes are utilised for a variety of other purposes including timber, medicine, tannins and gums. Various species of *Lonchocarpus* and *Derris* are the source of rotenone, which is used as an insecticide, fish poison or molluscicide. Some legume trees yield valuable resins, used in varnishes, paints and lacquers. The economic importance of the family is likely to increase as human pressure places greater demand on marginal land, since many legume species are characteristic of open and disturbed places and are thus well adapted to grow under poor conditions [9].

<table>
<thead>
<tr>
<th>Main edible legume seeds (grain legumes)</th>
<th>Latin name</th>
<th>World crop production*, metric tons $\times 10^{-3}$</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pulses:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1. Dry beans (<em>Phaseolus</em> spp. including several species now in <em>Vigna</em>):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney bean, haricot bean, pinto bean, navy bean</td>
<td><em>Phaseolus vulgaris</em></td>
<td>3819</td>
</tr>
<tr>
<td>Lima bean, butter bean</td>
<td><em>Vigna lunatus</em></td>
<td>1162</td>
</tr>
<tr>
<td>Adzuki bean</td>
<td><em>Vigna angularis</em></td>
<td></td>
</tr>
<tr>
<td>Mung bean, golden gram, green gram</td>
<td><em>Vigna radiata</em></td>
<td></td>
</tr>
<tr>
<td>Black gram, urd</td>
<td><em>Vigna mungo</em></td>
<td></td>
</tr>
<tr>
<td>Scarlet runner bean</td>
<td><em>Phaseolus coccineus</em></td>
<td></td>
</tr>
<tr>
<td>Rice bean</td>
<td><em>Vigna umbellata</em></td>
<td></td>
</tr>
<tr>
<td>Moth bean</td>
<td><em>Vigna aconitifolia</em></td>
<td></td>
</tr>
<tr>
<td>Tepary bean</td>
<td><em>Phaseolus acutifolius</em></td>
<td></td>
</tr>
<tr>
<td>2. Dry broad beans (<em>Vicia faba</em>):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Horse bean</td>
<td><em>Vicia faba</em></td>
<td>255</td>
</tr>
<tr>
<td>Broad bean</td>
<td><em>Vicia faba</em></td>
<td></td>
</tr>
<tr>
<td>Field bean</td>
<td><em>Vicia faba</em></td>
<td></td>
</tr>
<tr>
<td>3. Dry peas (<em>Pisum</em> spp.):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garden pea</td>
<td><em>Pisum sativum var. sativum</em></td>
<td>892</td>
</tr>
<tr>
<td>Protein pea</td>
<td><em>Pisum sativum var. arvense</em></td>
<td></td>
</tr>
<tr>
<td>4. Chickpea</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. Dry cowpea, blackeye pea, blackeye bean</td>
<td><em>Cicer arietinum</em></td>
<td>478</td>
</tr>
<tr>
<td>6. Pigeon pea, cajan pea, congo bean</td>
<td><em>Vigna unguiculata</em></td>
<td></td>
</tr>
<tr>
<td>7. Lentil</td>
<td><em>Cajanus cajan</em></td>
<td>103</td>
</tr>
<tr>
<td>8. Bambara groundnut, earth pea</td>
<td><em>Lens culinaris</em></td>
<td>199</td>
</tr>
<tr>
<td>9. Vetch, common vetch</td>
<td><em>Vicia subterranea</em></td>
<td></td>
</tr>
<tr>
<td>10. Lupins</td>
<td><em>Vicia sativa</em></td>
<td>99</td>
</tr>
<tr>
<td>11. Minor pulses;</td>
<td><em>Lupinus spp.</em></td>
<td>45</td>
</tr>
<tr>
<td>Lablab, hyacinth bean</td>
<td><em>Lablab purpureus</em></td>
<td></td>
</tr>
<tr>
<td>Jack bean, sword bean</td>
<td><em>Canavalia ensiformis, gladiata</em></td>
<td></td>
</tr>
<tr>
<td>Winged bean</td>
<td><em>Psophocarpus teragonolobus</em></td>
<td></td>
</tr>
<tr>
<td>Velvet bean, cowitch</td>
<td><em>Mucuna pruriens var. utilis</em></td>
<td></td>
</tr>
<tr>
<td>Yam bean</td>
<td><em>Pachyrhizus erosus</em></td>
<td></td>
</tr>
<tr>
<td><strong>Non-pulses</strong> (oil-crops):</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td><em>Glycine max</em></td>
<td>6209</td>
</tr>
<tr>
<td>Peanut</td>
<td><em>Arachis hypogaea</em></td>
<td>1874</td>
</tr>
</tbody>
</table>

*FAOSTAT data, 2004 [10].
The Food and Agricultural Organisation (FAO) of the United Nations defines pulses as annual leguminous crops yielding from one to 12 grains or seeds of variable size, shape and colour within a pod. This term is reserved for crops harvested solely for the dry grain and therefore excludes green beans and green peas, which are considered vegetable crops. Also excluded are crops which are mainly grown for oil extraction, like soybean and peanut, and crops which are used exclusively for sowing, like clovers. Pulses are important food crops due to their high proteins and essential amino acid content. Like many leguminous crops, pulses play a key role in crop rotation due to their ability to fix nitrogen.

Table 1 reports the list of legume crops according to FAO classification [10].

3. Grain legume proteins

Legume seeds accumulate large amounts of proteins during their development. Most of them are devoid of any catalytic activity nor they play any structural role in the cotyledonary tissue. They are stored in membrane-bound organelles, the storage vacuoles or protein bodies, in the cotyledonary parenchyma cells, survive desiccation in seed maturation and undergo proteolysis at germination, thus providing free amino acids, as well as ammonia and carbon skeletons to the developing seedlings. These seed proteins are termed storage proteins [11]. Legume seeds contain several comparatively minor proteins, including protease and amylase inhibitors, lectins, lipoxygenase, defence proteins and others, which for various reasons are relevant to the nutritional/functional quality of the seed. Some of them also seem to have adopted a storage role by virtue of their amount in the seed [12].

Proteins in legume seeds represent from about 20% (dry weight) in pea and beans up to 38–40% in soybean and lupin [13,14]. Therefore legume seeds are among the richest food sources of proteins and amino acids for human and animal nutrition. Traditionally, the classification of storage proteins is based on their solubility properties: albumins are soluble in water, globulins are soluble in salt water solutions and prolamins are soluble in ethanol/water solutions [15]. The latter ones are most prominent in cereal seeds. This old classification scheme still has an operative validity, specially in relation to the techno-functional properties of these proteins.

The most abundant class of storage proteins in grain legumes are the globulins. They are generally classified as 7S and 11S globulins according to their sedimentation coefficients (S). The 7S and 11S globulins of pea are named vicilin and legumin, respectively, so that the corresponding proteins of other seeds are often indicated as vicilin- and legumin-like globulins. The 7S proteins are oligomeric proteins (usually trimers). The 11S proteins are also oligomers, but usually they form hexamers [1]. Larger aggregates of 15–18S have also been reported for soybean legumin-like proteins [16]. Under dissociating conditions, both the 7S and 11S globulins liberate their constituent subunits. These polypeptide chains are naturally heterogeneous, being the selective pressure on them scarce. Heterogeneity is evident at both size and charge levels [17–19], and arises from a combination of different factors, including the multigenic origin of each storage globulin and the post-translational modifications of relatively few expression products [11,20]. The mutual contribution of these factors vary significantly intra- and inter-generically.

From the nutritional viewpoint, all legume storage proteins are relatively low in sulphur-containing amino acids, methionine, cysteine and tryptophan, but the amounts of another essential amino acid, lysine, are much greater than in cereal grains [21,22]. Therefore, with respect to lysine and sulphur amino acid contents, legume and cereal proteins are nutritionally complementary. The degree of mutual supplementation may also depend, however, on the contents of the second limiting amino acids, i.e., threonine in cereals and tryptophan in legumes. These essential amino acid deficiencies have traditionally been overcome by integrating legume-based dishes with cereal foods (pasta, rice, bread, etc.). However, amino acid composition only represents the potential quality of a protein food, being their bioavailability critical for the supply of amino acids in the diet. Indeed, the lack of availability may represent a serious hindrance for the full exploitation of these proteins. A number of experimental approaches, devised to assess the bioavailability of amino acids in foods, concurrently demonstrate that seed proteins have a lower overall nutritional quality than animal proteins. This can be related to their low content of sulphur-containing amino acids [23], the compact proteolysis-resistant structure of the native seed proteins [24] and the presence of anti-nutritional compounds, which may affect digestibility of proteins themselves and other components [25–27].

Legume seeds contain also a number of anti-nutritional compounds (ANCs) which can be of proteinous, i.e., hydrolase inhibitors and lectins, and non-proteinous nature. The presence of ANCs in crop plants is often the result of an evolutionary adaptation which enables the plant to survive and complete its life cycle under natural conditions. Indeed, due to their anti-nutritional or even toxic properties, various seed components have been shown to play a protective role against insects [28], fungi [29], predators and a number of stress conditions [30]. For example, hydrolase
inhibitors have proven to act as protective agents against insect attack [31,32] and their involvement in wound responses has received attention in a number of species [33]. The negative consequences of ANCs’ presence on the quality and safety of the food products reflect a typical anthropocentric vision. However, in the case of grain legumes, most, if not all, the putative nutraceutical compounds arise from the so called antinutrients, which explain why a reappraisal on their roles and effects is currently taking place [34].

Among the protein ANCs, seed hydrolase inhibitors play a major role, due to their spread diffusion in many legume grains, including pea, lentil, bean as well as soybean. Their anti-nutritional effect consists in the inhibition of various digestive enzymes, including trypsin, chymotrypsin, amylase [35]. However, their effect is usually manifest only if the seed or the flour are consumed uncooked, since heat denaturation, as the consequence of legume seed cooking, normally inactivates these proteins [36]. Once inactivated, the protein inhibitors may even play a positive nutritional role, due to their high content of sulphur-containing amino acids relative to the majority of the seed proteins [37]. The most characterized protein inhibitors are trypsin/chymotrypsin inhibitors of both the Bowman-Birk [38] and Kunitz type [39] and α-amylase inhibitors [40]. The effects of these inhibitors on the digestion processes are usually studied in vitro systems [38]. A relatively low level of inhibition is observed when purified pea TIs are evaluated using this system, compared to experiments where soybean TIs are used. Moreover, variation in pea protein digestibility has been observed among a set of pea lines that are near-isogenic and contain similar seed trypsin inhibitor activities, suggesting that seed compounds, other than inhibitors, may be of greater importance in affecting protein digestibility [41].

Seed lectins are another family of protein ANCs in grain legumes. In 1888, Stillmark reported that castor bean extracts were able to agglutinate red blood cells from different animal species [42]. These seeds extracts were named agglutinins and later on, in 1954, lectins [43]. Lectins are ubiquitous (glyco)proteins which exhibit specific and reversible carbohydrate binding activities. The specificity of a lectin is defined in term of the monosaccharide or simple oligosaccharide which inhibits the lectin-induced cell agglutination reaction. However, many, but not all, lectins have haemagglutinating activity [44]. Although lectins are similar to antibodies in their ability to agglutinate red blood cells, they differ in that lectins are not products of the immune system, their structures are diverse and their specificity is restricted to carbohydrates [45]. The toxicity of lectins is characterized by growth inhibition in experimental animals, and by diarrhoea, nausea, bloating and vomiting when injected in humans [46]. Heat processing can reduce the toxicity of lectins, but low temperature or insufficient cooking may not completely eliminate their toxicity [47]. Although the biological role of lectin in legume seeds is still controversial, there is evidence that lectins can be defence proteins against potential plant enemies. Since the majority of plant lectins exhibit a specificity against carbohydrates of animal origin, one can reasonably argue that plants use this type of protein as a defence against phytophagous invertebrates and herbivorous animals. Feeding trials confirm the deleterious effect of various plant lectins on insects and rats. However, due to their limited toxicity, lectins probably did not evolve as protectants of individual plants, but rather as resistance factors which are beneficial for the survival of the species [28]. Other physiological roles of lectins have been put forward, suggesting that their sugar-binding capacity may be multifunctional.

4. Beneficial effects of legume seeds

Grain legumes can hardly be found in the old books of traditional medicine as specific therapeutic agents. Some information can be found concerning minor and local species, specially in the eastern and far eastern countries (India and China). Other indications of phytotherapeutic use of legumes comes from the Mediterranean regions, where grain legumes have traditionally been consumed for centuries and constitute an element of the Mediterranean diet [48,49]. On the other hand, several suggestions of the role of pulses in the prevention of relevant diseases, typical of the affluent countries, are available and represent the basis for the healthy claims on legume seeds.

As reminded by Kushi et al. [50] the observation that diets low in meat and high in cereals and legumes are beneficial for health was noted at least as far back as the Old Testament in the first chapter of the Book of Daniel. More recent, but still vague, reports on pulse beneficial effects on man health and putative causative agents (rarely proteins) are summarised here below.

4.1. Cardiovascular disease (CVD)

The frequent intake of dry legumes, in parallel with a saturated-fat poor diet, can help controlling the lipid homeostasis and consequently reduce the risk of CVD. The legume high fibre content, their low glycaemic index and
the presence of minor components, such as phytosterols, saponins, oligosaccharides, etc., are considered the main responsible agents for this property.

4.2. Diabetes

Because of the low glycaemic index (GI) and the high content of undigestible fibres, dry legumes are claimed to help glycaemic control in diabetic individuals. Moreover, dry legumes may contribute to prevent insulin-resistance, which represents the prodrome to type II diabetes.

4.3. Digestive tract diseases

The accelerated transit of the digested food in the intestinal tract and its final excretion play a number of positive effects, decreased re-absorption of cholesterol, incomplete starch digestion, lowering of fermentation processes. These factors have been considered beneficial in the prevention of cancer, specially colon cancer. Legumes are thought to play an important role in this respect.

4.3. Overweight and obesity

Despite their content of lipids, starch and proteins, dry legumes are claimed to help maintaining a regular body weight, thanks to their great satiety effect, thus limiting the overall food daily intake. Various seed components have been claimed to bring about this effect.

5. Demonstrated beneficial effects of selected legume proteins

Aim of this review being to show examples of a modern and rational understanding of the actual beneficial effects of legume protein components in the human diet, the biological activities of specific proteins, considered to be responsible for at least part of the observed effects, will be described. The main examples are also reported in summary in Table 2.

<table>
<thead>
<tr>
<th>Peptide/protein</th>
<th>Legume seed</th>
<th>Intrinsic activity</th>
<th>Biological activity</th>
<th>Literature references</th>
</tr>
</thead>
<tbody>
<tr>
<td>7S globulin α’-chain</td>
<td>Soybean</td>
<td>n.a.</td>
<td>—Up-regulation of LDL-receptors</td>
<td>57–63</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>—Plasma cholesterol and triglyceride reduction</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>—Anti-atheromatous activity</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Plasma cholesterol and triglyceride reduction</td>
<td>60, 64–68</td>
</tr>
<tr>
<td>Undefined storage proteins</td>
<td>Faba bean, lupin and others</td>
<td>n.a.</td>
<td>—Anti-cancer</td>
<td>81–88</td>
</tr>
<tr>
<td>BB serine–protease inhibitor</td>
<td>Soybean, pea and others</td>
<td>Trypsin/ chymotrypsin inhibitor</td>
<td>—Anti-inflammatory</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>—Anti-obesity</td>
<td>91</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>—Anti-degenerative and autoimmune diseases</td>
<td>92–93</td>
</tr>
<tr>
<td>α-amylase inhibitor</td>
<td>Various sources</td>
<td>α-amylase inhibitor</td>
<td>—Weight control</td>
<td>101</td>
</tr>
<tr>
<td>Conglutin γ</td>
<td>Lupin</td>
<td>n.a.</td>
<td>—Obesity</td>
<td>102</td>
</tr>
<tr>
<td>Lectins</td>
<td>Various sources</td>
<td>Glycosyl moieties binding</td>
<td>—Hypoglycaemic</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>—Hypocholesterolaemic</td>
<td>66</td>
</tr>
<tr>
<td>ACE inhibitor peptides</td>
<td>Soybean</td>
<td>Alkalase treatment/fermentation of soybean proteins</td>
<td>—Anti-cancer</td>
<td>95–96</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>—Immuno-modulation</td>
<td>94</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Hypotensive</td>
<td>119—120</td>
</tr>
</tbody>
</table>

n.a.: not assessed.
5.1. Lipid homeostasis control and hypocholesterolaemic effects of soybean proteins

In October 1999, the U.S. Food and Drug Administration (FDA) approved a health claim that allowed food label claims for reduced risk of heart disease on foods that contain \( \geq 6.25 \) g of soybean protein per serving [52]. In particular, a daily soybean protein intake of 25 g was considered beneficial, based on a number of previous clinical observations. Although the substitution of animal with plant proteins in the diet of hypercholesterolaemic individuals is known to be associated with a significant cholesterol reduction since long [53] and the identification of the responsible molecules, including phytoestrogens, dietary fibre, protein components, etc., is still matter of debate, this claim is one of the rare examples of an official acknowledgement of a dietary protein beneficial effects on health. However, the amounts of soybean proteins needed to trigger the biological activity are extremely large from the dietetic point of view. This clearly hampers almost any practical use of soybean proteins, except in specific serious hypercholesterolaemia cases. Therefore, the identification of the active molecule(s) has strongly been pursued as a strict requirement for the design of suitable intervention protocols, in the last years. The first in vivo evidence of the involvement of the 7S globulin family of soybean storage proteins was obtained in 1992 by Lovati et al. [54]. In this study, a direct effect on the reduction of plasma cholesterol levels in rats of 35% was observed with dosage and effects comparable to those obtained with clofibrate. The results also showed a statistically significant decrease of triglyceride levels in rats. These findings were confirmed by studies on the stimulation of low-density lipoproteins (LDL) receptors and degradation of LDL in cultured hepatocytes [55]. Later on, an evidence of the involvement of one of the 7S globulin subunits, namely the \( \alpha' \) subunit, was indirectly shown by the lack or reduced activity of a soybean cultivar naturally devoid of this polypeptide chain [56]. Further studies [57] confirmed these previous findings, by showing the up-regulation of LDL receptors by the \( \alpha' \) subunit. The final in vivo direct demonstration of the key role played by the \( \alpha' \) subunit on the cholesterolaemic and triglyceridaemic levels came later, thanks to the isolation of relatively large amounts of this subunit from the 7S globulin oligomer [58]. This achievement was based on the observation that the N-terminal region of the \( \alpha' \) subunit, the so-called extension region, differed from the corresponding region of the \( \alpha \) subunit being richer in histidine residues (SwissProt Database accession numbers of \( \alpha' \) and \( \alpha \) subunits: P11827 and P13916, respectively). On this basis, a preparative metal affinity chromatography column, was used to isolate the \( \alpha' \) subunit in gram amounts, making it possible to test the isolated subunit in hypercholesterolaemic animal models as well as in cell assays [58]. The results are reported in Fig. 1. These results, by showing that the \( \alpha' \) subunit oral administration to rats significantly reduced plasma cholesterol and triglyceride levels, confirmed in vivo the results obtained with soybean proteins, 7S globulin and \( \alpha' \) subunit, in isolated cell systems, ruled out any isoflavone effect [59] and substantiated the role of a legume dietary protein in the management of dislipidaemia. In addition, the up-regulation of the \( \beta-VLDL \) receptors in liver cells from hypercholesterolaemic rats in response to oral treatment with this polypeptide was demonstrated. Nonetheless, since the purification procedure devised is not easy to scale up, by implying a denaturation step to
dissociate the 7S globulin oligomer, the possibility of cloning and expressing in proper host organisms the active subunit or a fragment thereof, bearing the biological activity, is currently being investigated in the author’s laboratory.

Although the mentioned results represent a relevant breakthrough in the utilisation of a dietary protein for the control of cholesterol and triglycerides haematic levels, still the mechanism of action of this protein is not understood. From a recent study, it appeared that soybean protein in the diet can also increase the size of LDL, a known protective effect against arterial diseases [60]. Moreover, the interaction of the soybean 7S globulin with thioredoxin 1 and cyclophilin B, two cell protein components involved in the protection from oxidative stress, was demonstrated [57].

Due to the complexity of the metabolic and regulatory pathways involved, further research activities with suitable models and experimental approaches are needed to identify underlying mechanism(s).

All these direct evidences support the epidemiological association between soybean protein intake and reduced cardiovascular risk [61]. Indeed, a positive effect of soybean proteins on carotids lesions in high fat diet fed rabbits [62] and a global anti-atheromatous effect in mice [63] suggest a link between soybean protein consumption and atherosclerosis reduction.

Due to the potentially great impact of these studies on human health, specially in the affluent countries, several researches in many areas and with different experimental approaches are currently ongoing. In this context, also the involvement of pulses other than soybean in the control of lipidaemic homeostasis has been considered [60,64,65]. In particular, a report on the reduction of plasma total and LDL-cholesterol induced by lupin proteins in rats on a high fat diet has appeared [66]. In attempt to identifying the putative responsible molecule, conglutin γ, a lupin protein which will be extensively mentioned in the following paragraph, has been put forward. Previously, another legume seeds, namely faba bean (Vicia faba) was proved to have a beneficial impact on lipid profiles. In human studies, Weck et al. [67] demonstrated that in hypercholesterolaemic subjects, faba bean proteins had a cholesterol-reducing efficacy comparable to that of soybean protein. This effect was later confirmed on hypercholesterolaemic rats by Macarulla et al. [68].

5.2. Glycaemic control of a lupin protein

Some carbohydrate-rich foods induce less post-ingestive hyperglycaemia–hyperinsulinaemia than others [69]. Consequently, the so-called glycaemic index (GI) of a food is based on the post-prandial blood glucose response compared with a reference food. Pulses are low GI foods in force of their starch peculiar composition [70]. However, the following example refers to a legume grain which does contain only traces of starch, suggesting that the hypoglycaemic effect, which is described further on, is due to a specific component other than starch. The legume seed in question is lupin, a well known, though under-exploited, protein-rich legume of the Mediterranean area. As mentioned in a previous paragraph, lupin seeds are among the very few edible grain legumes to which a medicinal property has been ascribed. In particular, an anti-diabetic activity of toasted lupin seeds was described [71]. In the attempt of identifying the active principle responsible of the glucose controlling capacity attributed to a lupin seed component, a study on a specific lupin protein, named conglutin γ, was initiated. Conglutin γ was considered a good candidate on the basis of previous finding that an homologous soybean seed protein, named Bg7S, was found to display a binding capacity to some small regulatory proteins, including insulin [72]. However, these precursor studies primarily focused on the modalities and possible physiological role of the interaction between the soybean protein and an endogenous regulatory peptide, thus neglecting the potential effect(s) of the soybean protein on the human body carbohydrate metabolism. Due to the amino acid sequence similarity (63%) between soybean Bg7S (SwissProt Database accession number: P13917) and lupin conglutin γ (SwissProt Database accession number: Q9FSH9), a molecular and metabolic study on the latter protein was recently undertaken. The isolated lupin protein was found to bind insulin in vitro, by using both affinity chromatography on an insulin-bound matrix and Surface Plasmon Resonance (SPR). These techniques allowed the determination of the quantitative binding parameters, in particular the kinetic and thermodynamic parameters, i.e., $k_{on}$ of $2.7 \text{M}^{-1}\text{s}^{-1}$, $k_{off}$ of $2.0 \text{s}^{-1}$ and $K_d$ of $7.2 \times 10^{-5} \text{M}$, respectively, and showed that the interaction is strongly affected by small ionic strength changes, not so much by the pH and requires the native structure of the protein to take place. In parallel, suitable amounts of isolated protein were tested in glucose overloading trials on rats. The results, reported in Fig. 2, clearly showed a significant decrease of the under-curve area of time course of glycaemic concentration with an effect similar to that of metformin at about half the dose of the lupin protein [73,74]. Also in this case, as with soybean protein hypocholesterolaemic effects, mechanism(s) by which the effect of the lupin protein is exerted is(are) not understood. Studies on both the biological activity of lupin conglutin γ...
and its metabolic effects are currently ongoing. Provided these first results will be confirmed also in human intervention studies, this lupin dietary protein candidates as a possible anti-diabetic drug, specially in control of pre-clinical diabetes, as suggested in the traditional phytotherapy.

Although anti-diabetic activity has also been described in other legume crops, the role of components other than proteins has been evoked, such is for a minor local legume grain, fenugreek (*Trigonella foenum graecum*) [75]. In this case, the biological activity was ascribed to the galattomannan-rich soluble dietary fibre and/or to a modified amino acid, i.e., 4-hydroxyisoleucine (4-OH-Ile)[76]. A general claim on the anti-diabetic role of legume seed α-amylase inhibitors has recently been published[77]. A protective role of *Phaseolus vulgaris* pod extracts on changes in the fatty acid composition in experimental diabetes of rats was also described [78].

5.3. Anti-carcinogenic effects of legume proteins (protease inhibitors and lectins)

Among potential protective micro-components against cancer which, to various extent, can be present in pulses, are included protease inhibitors, saponins, phytosterols, lectins and phytates [79]. However, according to Mathers [79] despite the fact that at least 58 studies (up dated to 1997) have reported results for intakes of pulses and cancer risk, it will be very difficult, using conventional epidemiological tools, to ascertain the quantitative contribution made by pulses to cancer risk. Other conclusions are more drastic: overall, the epidemiologic data on breast cancer reduction upon soybean intake are inconclusive[80]. Nevertheless, it is specially in this area that studies on the beneficial effects against cancer of specific and isolated legume protein components have been carried out since long. In particular, two classes of legume proteins seem to play a role: the protease inhibitors and the lectins, both already cited amongst the legume ANCs in 3. Grain legume proteins.

Proteases are considered key players in a wide range of biological processes and misfunctioning of certain proteases has been related to diseases, including cancer progression [81]. Therefore, a control of this activity by protease inhibitors appear to closely relate to the capacity of preventing or blocking certain tumoral pathologies. Studies with soybean serine protease inhibitors of the Bowman-Birk inhibitor (BBI) family have provided evidence of an arrest of certain mammalian tumors [82]. More recently, the anti-proliferative effects on human colon cancer cells of two recombinant wild-type Bowman-Birk inhibitors from pea seeds have been reported [81]. The measured effect was much greater than with soybean BBI. According to Clemente et al. [81] these results could be explained following the identification of target proteases and by dissecting the apoptotic and mitotic processes which are affected by the inhibitor inside the test cells. BBIs have been shown to be effective by both in vitro and in vivo approaches [83,84]. Other seed protease inhibitors have demonstrated their anti-tumoral efficacy [85,86]. More recently, studies on specific cancer forms directly in humans have been carried out [87,88].

BBI is a double-headed inhibitor usually capable of inhibiting both trypsin and chymotrypsin. It has been proposed [81] that the anti-chymotryptic activity is more effective that the anti-tryptic activity in the suppression of carcinogen-
induced transformation. Therefore, a mutagenesis program on this protein molecule aimed at converting the inhibitor to a double chymotrypsin form could be advantageous for an increased biological activity. It has also been claimed that the potential beneficial effects require the native protein conformation and therefore would not be attained after proper cooking of legumes [89]. On the other hand, incomplete denaturation of the BBI resistant protein structure cannot be excluded, specially upon legume-based dish domestic preparation. This can be the basis for an effective preventive activity attributed to the regular legume intake.

Besides the anti-carcinogenic effects, BBIs also showed anti-inflammatory activity, by inhibiting the inflammation-mediating protease. In this context, a reduction of ulcerative colitis in mice was reported [90]. More recently, a number of patents on the use of BBIs for combating obesity [91] and even degenerative and autoimmune diseases, including multiple sclerosis, Guillain Barre syndrome [92] and, in general, skeletal muscle atrophy [93] have appeared.

Since long, lectins are considered as promising biologically active molecules. However, their observed numerous effects, including induction of small intestine hyperplasia, changes in the intestinal flora, immuno-modulating activity, interference with hormone secretion and access to the systemic circulation [94] have hampered their straightforward utilisation for specific medical uses. Nevertheless, a number of reports on various animal models conclusively showed that lectins may limit tumour growth by promoting gut epithelium hyperplasia or through other effects [95,96].

5.4. Weight control/obesity

Obesity is a complex disease with serious medical consequences [97]. Nevertheless, obesity is growing in many countries, including some beyond suspicion, like China and other far eastern countries, and also among the child population in the affluent countries. In some regions the prevalence can reach 50% of the adults. Dietary factors seem to play a crucial role in this trend, along with other aspects of life-style, e.g., inactivity [98]. Various studies suggest that protein is more satiating than carbohydrate or fat (see literature cited in Ref. [99]). In this respect, an increased legume-protein intake may have weight-loss benefits, also thanks to the lower extent of kidney workload of plant proteins with respect to animal proteins [100]. However, a specific direct action of grain legume α-amylase protein inhibitors has been considered for its potential use in the prevention and therapy of obesity and diabetes. To this aim, studies carried out on normal, obese and diabetic subjects by oral administration of wheat α-amylase inhibitor, have shown a delayed carbohydrate absorption, a reduction of peak postprandial plasma glucose concentrations with no malabsorption nor other symptoms [101]. More recently, the binding parameters of the association between wheat α-amylase inhibitor and porcine pancreas α-amylase and the high thermal stability of the protein inhibitor [102] have confirmed the potentiality for its use as a nutraceutical molecule. Indeed, some patents concerning the use of food preparations containing suitable amounts α-amylase inhibitors from various sources for the obesity control and the prevention and treatment of diabetes have recently appeared [103,104].

5.5. Mineral micronutrient vehiculation

Although legume seeds notoriously contain several minor constituents, such as phytaetes, which may actively chelate metal ions, thus reducing their bio-availability, grain legumes are also considered fairly good sources of mineral micronutrients [80]. Since one of the main problems concerning a suitable supply and absorption of micronutrients is their bio-availability, a proper vehiculation through the intestinal tract is essential. Food proteins and their peptide fragments can effectively act as metal biological carriers. An example can be the same lupin protein shown to possess glycaemia controlling properties, namely conglutin γ, which was found to be able of interacting with a number of metal ions, including Zn++, Cu++ and, to a minor extent, Cr+++., Fe++, Co++, Ni++, Cd++, and Sn++. The binding was shown to be related to the numerous histidine residues concentrated in the large protein subunit [105], but no further studies on the intrinsic activity, if any, or the role of the protein/metal complexes were performed.

The question of protein metal vehiculation is still open, because several other factors may affect this carrier property. For example, evidence that the heat-induced insolubilisation process adversely affects not only protein but also Cu and Fe bio-availability from legumes and that polyphenols are likely to be a major inhibitor on absorption has been provided [106,107]. As in most cases, due to the complexity of the digestion and absorption mechanisms, it is practically impossible to fulfil in vivo all the optimal conditions and requirements that specific studies on isolated model systems have evidenced.
6. Synergistic effects

One of the major problems set up by the association of nutrition and health, relates to the insuperable complexity of the phenomena and mechanisms involved. In other words, both disease and prevention/treatment rarely can be afforded with a single approach. The holistic nature of most diseases is particularly evident especially for those which are nutrition-related. For example, the so-called metabolic syndrome is characterized by a group of metabolic risk factors in one person. They include: obesity, atherogenic dyslipidaemia, raised blood pressure, insulin resistance or glucose intolerance, pro-thrombotic and pro-inflammatory states and other less obvious conditions. The underlying causes of this syndrome are overweight/obesity, physical inactivity and genetic factors. People with the metabolic syndrome are at increased risk of coronary heart disease, other diseases related to plaque build up in artery walls (e.g., stroke and peripheral vascular disease) and type 2 diabetes. In this context, it would seem logical to consider that also an holistic intervention on all or most causes is required for the effective prevention and treatment of such multi-factorial diseases. The cited general guidelines by various scientific boards on the recommended intake of fresh fruit, vegetables, legumes, cereals, etc. stand in this line.

A key question concerns the different effect, if any, of a whole seed consumption vs. legume seed single component. The whole grain intake may deliver more nutrition and health benefit than the sum of its part [108]. Indeed, it has been shown that protein, fibre, starch, vitamins, minerals and other component of pulses (oligosaccharides, isoflavones, phospholipids, saponins, and antioxidants) all probably contribute to the cardiovascular protective effects of pulses [109]. Therefore, due to the synergistic effects of several components, it is likely that various pulses may display similar beneficial effects, as reviewed by Anderson and Mayor [61]. These authors concluded that “intake of pulses, with their low glycaemic index and mineral content, has favourable effects on blood pressure, glycaemic regulation and weight management. The cardio-protective effects of pulses are thus multi-factorial. The available evidence gives confidence in recommending regular intake of pulses to reduce risk of cardiovascular disease” [61]. From this point of view, the synergistic effects of several legume seed components support the conclusion that seeds should be consumed as a whole food in order to take advantage of their beneficial effects, as directly shown for faba bean protein isolates and whole seeds cholesterol decreasing effects [68].

On the other hand, the need of identifying single bio-active molecules in foods is acute, since it helps understanding the underlying mechanism(s), designing suitable intervention protocols and also calibrating the dose/response of the treatment. From the side of protein involvement, only in few mentioned cases, a specific grain legume protein component has been proved to be responsible for a specific dietary effect, others can only be considered as putative bio-active molecules, while it is relatively common to ascribe a property to a synergic action of various protein and non-protein components.

7. A scarcely understood key question: transit, processing and absorption of biologically active proteins in the intestinal tract

Considering the nutraceutical role of proteins raises a general question on their metabolic fate during the transit in the gastro-intestinal tract. Indeed, the extent of food protein enzymatic proteolysis has a dramatic impact on the occurrence of the biological effect. Various angles are possible: if the intact protein is needed to express bio-activity, the digestion process can be seen as a limiting factor; if the activity is brought about by peptide fragments liberated from the food protein, proteolysis is clearly a prerequisite for the expression of the biological activity (see the paragraph on cryptic bio-activities). Moreover, some (poly)peptides, which are normally digested by the gut proteolytic enzymes have been shown to be protected, if administered together with alternative protein substrates, such as casein [110,111]. Some plant proteins are intrinsically resistant to proteolysis not only in vitro, but also in vivo. This is quite obvious for the protease inhibitors, but also survival of lectins in the gut was shown to range from 20% to >90% [112]. SDS-PAGE characterisation of the gastro-intestinal digested material arising from the seed globulins and amino acid analysis of undigested proteins of whole cooked common bean and faba bean suggested that are the structural properties of the storage proteins, rather than their binding to polyphenols, that mainly determines the extent of protein aggregation on autoclaving and may therefore be responsible for their low digestibility [113]. The beneficial effects of various legume (and non legume) resistant proteins on pathological conditions such as hypercholesterolaemia, constipation and even colon tumorigenesis, have been reviewed by Kato and Iwami [114]. According to these authors, the mechanism would be related to both binding capacity of
molecules, like bile acids, and a positive influence on enteric fermentation, thus acting similarly to resistant starch and other polysaccharide undigestible compounds.

In 1997, the French researcher Yves Boirie and colleagues [115] introduced the terms “fast” and “slow” dietary proteins, in analogy to fast and slow starch, to describe the effect of two different proteins intake on the body protein synthesis, breakdown and oxidation. A relevant effect on body metabolism was shown to be determined by the different speed of amino acids liberation from the food proteins during digestion. Partial proteolysis in the gut not only determines altered kinetics of amino acid liberation and absorption, but also may affect several other intestinal functionalities, such as local intestinal cell modifications, changes in absorption kinetics and mechanisms, internalisation of peptides and occasionally proteins or major fragments of these.

Absorption of intact proteins occurs only in specific circumstances, since very few proteins get intact through the gauntlet of soluble and membrane-bound proteases. Moreover, normal enterocytes do not have transporters to carry proteins across the plasma membrane and they certainly cannot permeate tight junctions. One important exception to these general statements is that for a very few days after birth, neonates have the ability to absorb intact proteins. This ability, which is rapidly lost, is of immense importance because it allows the newborn animal to acquire passive immunity by absorbing immunoglobulins in colostral milk. In adults, clinical conditions known to be associated with or caused by increased/ altered intestinal permeability include: inflammatory bowel disease (Crohn’s and ulcerative colitis), celiac disease (gluten intolerance), inflammatory joint disease, rheumatoid arthritis (and maybe any kind of arthritis); food allergy, anything from acne, psoriasis, eczema, and intestinal disorder to chronic fatigue syndrome and temporo-mandibular joint syndrome (TMJ), alcoholism; ankylosing spondylitis, Reiter’s syndrome (urethritis, arthritis and inflammation of the bowel, more common in men), malabsorption, malnutrition, accelerated aging, giardiasis, intestinal infections, endotoxaemia, schizophrenia, chronic fatigue syndrome and temporal-mandibular joint syndrome (TMJ), alcoholism; ankylosing spondylitis, Reiter’s syndrome (urethritis, arthritis and inflammation of the bowel, more common in men), malabsorption, malnutrition, accelerated aging, giardiasis, intestinal infections, endotoxaemia, schizophrenia, thermal injury, Non-Steroidal Anti-Inflammatory Drug (NSAID) enteropathy, and being HIV positive.

Therefore, the question is extremely complex, since it concerns several basic and applied aspects, from biochemistry to nutrition, from physiology to pathology, which are up to now scarcely understood.

8. Cryptic bio-activities

As mentioned above in the case of soybean protein and hypocholesterolaemic activity, not only the intact α’ subunit but also smaller peptides produced in vitro by enzymatic hydrolysis of the 7S globulin appeared to have the same effects on cultured hepatocytes as the complete 7S globulin [116]. This finding shows that bio-active small peptide(s) can be somehow hidden in the polypeptide chain and their activity is made evident upon digestion in the intestinal tract and subsequent absorption [117]. This issue is of course strictly related with the question of the endogenous proteolysis of food proteins brought about by the gastro-intestinal enzymes, as mentioned in the previous paragraph. It has often been shown that proteins and peptides originating from incomplete hydrolysis of food proteins demonstrate biological effects in various test systems. The bioactivity of peptides which are latent until released from a protein by enzymatic proteolysis are called cryptic activities [118].

However, in legume seed area, relatively few examples of cryptic bio-active peptide fragments have been described so far. Angiotensin converting enzyme (ACE, EC 3.4.15.1) inhibitory peptides which give rise to a significant systolic blood pressure drop, have been produced by enzymatic treatment of non-genetically modified soybean and have also been found in fermented soybean preparation [119, 120]. An immuno-stimulating peptide isolated from an enzymatic digest of soybean protein prevented cancer chemotherapy-induced alopecia [121].

The case of the lectin-like breakdown product of a 7S lupin globulin, where limited proteolysis liberated a 20 kDa protein fragment with lectin-like activity which was not detectable before digestion [122], is particularly intriguing. Indeed, the proteolysis of the 7S globulin occurred endogenously during lupin seed germination.

As it can be noted, this finding opens an enormous number of perspectives relative to the existence of many different biological activities not only among the various plant sources, but also in different plant ontogenesis phases.

9. GM legume seeds and nutraceutical properties

Genetic modification of organisms is still far from being applied in the nutraceutical sphere, primarily because little is known about the role and mechanisms of action of the best part of bio-active proteins/peptides and on the molecular requirements for their proper administration, so far. However, a growing interest and use of the genetic
manipulation to produce recombinant proteins of high nutritional/therapeutic value in the food legumes is foreseen.

One of the few examples of GM-mediated introduction of bio-active peptides into food proteins is represented by the fusion of heterologous active peptides into a canonical storage protein. Ovokinin, a potent hypotensive peptide designed on the basis of a vasorelaxing peptide derived from ovalbumin, was introduced in homologous sites in the extension domain of the soybean 7S globulin α′ subunit \[123\], the same protein shown to be involved in the cholesterol reduction. More recently, a re-designed genetic modification with up to four ovokinin peptides introduced into the soybean protein showed from 800 to 2000 fold the activity of ovalbumin. This achievement resulted from the introduction of more peptide copies, and from the engineering of the proteolytic cleavage sites which give rise to a more efficient release of the active peptides \[124\]. This approach illustrates the extraordinary potentialities of this technique as a tool to introduce heterologous peptides into traditional plant foods. This second generation of genetically modified foods is expected to have a more positive impact and acceptance on consumers, since the consequent beneficial effects on human health can be immediately and measurably perceived.

10. Conclusions and prospects

The nutritional key role of grain legumes is unquestionable, due to the massive presence of macro and micro nutrients. Among these nutrients, proteins play a relevant role in consideration of their amino acid composition which can easily be balanced in the diet. In the frame of a reappraisal of the effects that grain legume components may have on human well-being, widely accepted claims on their beneficial activity in the prevention and treatment of various diseases has been put forward. Altogether, these claims strongly support the regular dietary intake of grain legumes as one of the ways to a healthy life.

Attempts to disclose the role of isolated legume components not always have produced the desirably unequivocal results. The reasons for this outcome are the complexity of the physiological processes involved: the multi-factorial nature of the diseases that grain legumes are thought to prevent or treat; the synergy between bio-active components, which often results in a greater global effect than that determined by the single component; the variability of the experimental approaches and conditions, which prevent the standardisation and comparison of the results and others. All this has not prevented to evidence relevant biological activities among the grain legume protein constituents in the last years. Specific proteins, and therefrom peptides, have unequivocally been shown to play a role in plasma lipid and glucose homeostasis, inhibition of hydrolytic enzymes involved in a number of critical physio-pathological phenomena, blood pressure control and immuno-modulation. All these basic functions are more or less directly related to a number of diseases, most of which are typical of the affluent countries, including cardio-vascular diseases, diabetes, cancer, obesity, hypertension, immunity-related diseases. Therefore, the general claims are receiving solid scientific support in a growing number of cases.

Nevertheless, many efforts and further studies are needed in order to disclose the mechanism(s) underlying the grain legume proteins/peptides effects; to identify and characterise novel biological activities often “hidden” inside the polypeptide chains, to establish clear dose/response relationships in order to calibrate the preparation and use of nutraceutically enhanced foods. As parallel or further steps, the biotechnological approaches can be extremely useful as cognitive tools and in novel more effective biologically active protein molecules design.

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