Chapter 17

Soybean: Non-Nutritional Factors and Their Biological Functionality

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1. Introduction

Legumes are important for the diet of a significant part of the world´s population; they are a good source of protein, carbohydrates, minerals and B-complex vitamins. In this sense, the soybean is an important legume because it has a high protein (35-48%) with a nutritionally balanced amino acid profileso their products are commonly used as a source of vegetable protein worldwide and a great proportion of high-quality oil (15-22%)[1].

The accessible price and stable supply are favourable factors for legumes to emerge as an important source of protein for human food [2]. However, the nutritional value of soybeans is lower than expected due to the presence of various non-nutritive compounds that hinder or inhibit the uptake of nutrients and produce adverse physiological and biochemical effects in humans and animals; since these could be toxic in some cases, they are referred as anti-nutritional factors [3, 4].

Recently it has been found that legumes, in the appropriate proportion, may have a beneficial role for health. It seems clear that, in many cases, the same interaction that causes legumes to be considered as anti-nutrients is responsible for its beneficial effects. Thereby,these compounds are called non-nutritional compounds or nutritionally bioactive factors, because while they lack nutritive value, are not always harmful [5]. Available data indicate that the balance between harmful and beneficial effects of these compounds is a function of concentration, exposure time and interaction with other dietary components. However, the threshold concentration at which the beneficial and harmful effects occur has not been evaluated in most cases [6].Moreover, they are compounds that do not appear

equally in all legumes, and their physiological effects in humans and other animals are different as well [7, 8].

These compounds have an important role in secondary metabolism of legumes, as reserve compounds for the biosynthesis of endogenous compounds, which accumulate in seeds and are used during the germination process, and as mechanisms of defense against bacteria, viruses, fungi, insects and animals [9].

From a biochemical point of view, these compounds have diverse nature. They may be proteins (protease inhibitors, α-amylase inhibitors and lectins), carbohydrates (α-galactosides, vicine, convicine, saponins), non-protein amino acids (L-DOPA, β-ODAP), polyphenols (condensed tannins, isoflavones), alkaloids, inositol phosphates, etc., so their extraction and quantification methodology is very specific. In soybeans, the non-nutritional factors are mainly; inositol phosphates, saponins, protease inhibitors, isoflavones, lectins, oligosaccharides and taninis[10, 11].

1.1. Phytic acid and inositol phosphates

Phytic acid (myo-Inositol hexakisphosphate or 1, 2, 3, 4, 5, 6-hexakis dihydrogen phosphate myo-Inositol), also abbreviated as $InsP_6$ or IP_6, is the main form of storage of phosphorus and inositol in seeds of cereals, legumes and oilseeds. However for humans and monogastric animals phosphorus is not available in that form, because these are not provided with sufficient activity of endogenous phosphatases (phytases) that are capable of releasing the phosphate group from phytic acid or inositol phosphates lighter phosphorylated [12].

This molecule is formed from the esterification of phosphate groups to each of the six hydroxyl groups in a molecule known as myo-Inositol (Figure 1). Usually, it represents 65 to 85% of total phosphorus in seeds while forming insoluble salts with mono and divalent cations. By releasing H+ ions from the phosphate groups, allows the molecule to interact with the ions Mn^{2+}, Fe^{2+}, Zn^{2+} and K^{+} to produce the corresponding salts, which are known as phytates. The name phytin has been used to designate a mixture of salts with Ca^{2+} and Mg^{2+}. Phytates and phytins usually bind to proteins in the protein bodies, the latter are membrane-limited structures where storage proteins are deposited. Salts of phytic acid are accumulated in seeds during the maturation period and are distributed uniformly in the cotyledons and embryonic axis in legumes [13, 14].

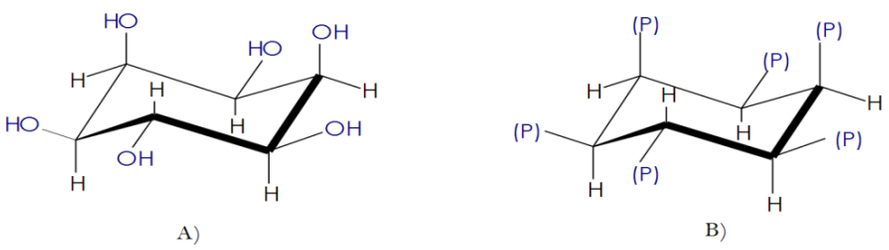

Figure 1. A) Chemical structure of myo-Inositol, B) Phytic acid structure (P) = H_2PO_4 [15]

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In the soybean (*Glycine max*) phytic acid is uniformly distributed in the cotyledons, in the same way as in most legumes, probably as a soluble potassium phytate, which constitutes approximately 1.5% of the total weight of the cotyledon. One gram of soybeans contains about 9.2-16.7 mg of phytate, which represents 57% of organic phosphorus and 70% of total phosphorus [16, 17].

1.1.1. Synthesis and Function

Phytic acid is sythesized from 1D-myo-Inositol 3-phosphate (Ins_3P_1); in turn, the latter is formed from D-glucose-6-phosphate by action of synthase Ins_3P1, and from myo-Inositol by action of myo-Inositol kinase; this reaction represents the first step in the metabolism of inositol and in the phytic acid biosynthesis. Subsequently, the phosphatidylinositol kinases catalyze the gradual phosphorylation of Ins_3P_1 to produce myo-Inositol di-, tri-, tetra-, penta- and hexaphosphate (Figure 2) [14, 18, 19].

During germination, phosphorus and cations are released from phytates by the increased activity of an enzyme called phytase, then, they become available for use during the seedling growth. The enzyme phytase (myo-Inositol-hexaquisfosfatophosphohydrolase) is capable of sequentially hydrolyzing phytic acid to myo-Inositol, which produces intermediate products with a lower number of phosphate ester groups (IP_5, IP_4, IP_3, and possibly di- and mono- phosphateinositols) and inorganic phosphate [20, 21].

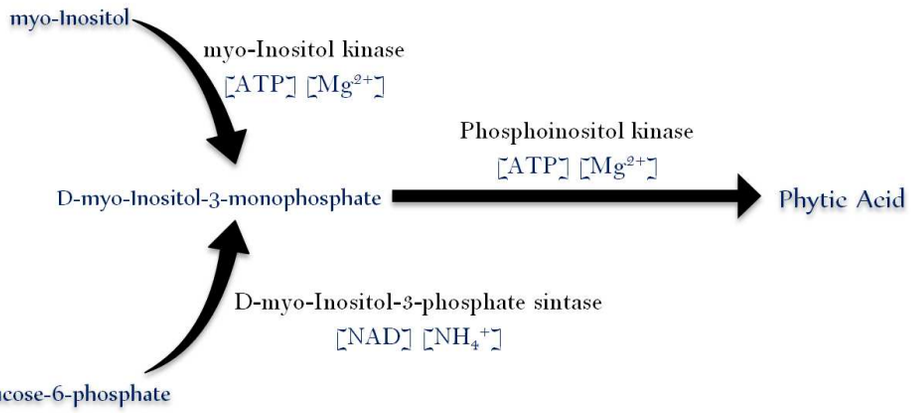

Figure 2. Biosynthesis of phytic acid [14].

A clear role of phytic acid in the seed tissues metabolism is the storage and recovery of phosphorus, minerals, and myo-Inositol during germination and growth [22]. Another physiological functions of phytic acid in plants, is the inhibition of the metabolism, since, by binding to multivalent cations required for cellular processes, the metabolism is slower, so it could be a latency-inducing molecule. Furthermore, the antioxidant capacity of phytic acid increases the time of seed latency, as it prevents lipid peroxidation [18, 23].

As well, phytates and also less-phosphorylated forms of phytic acid regulate diverse cellular functions such as DNA repair, chromatin remodelling, endocytosis, nuclear export

of mRNA, and is an important hormonal marker for the development of seedlings and seeds [24-28].

Less-phosphorylated molecules of myo-Inositol are presentin free form in nature, in small amounts, as transient intermediates in biochemical reactions. The mono-, bi-, tri- myo-Inositol phosphates are important components of a group of phospholipids, known as phosphoinositides, which are present in many plants and animal tissues [18]. Raboy (2009),reported a very detailed description of the synthesis and metabolism of phytic acid and myo-Inositol phosphates in plants (Figure 3).

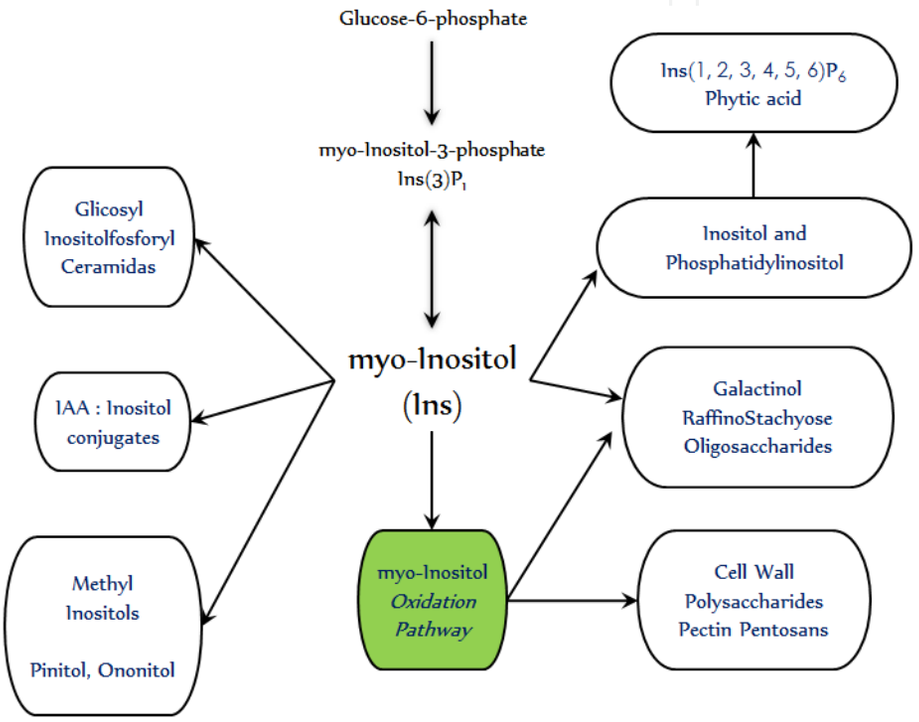

Figure 3. Pathways in plant biology that utilize myo-Inositol [29].

1.1.2. Bioavailability of minerals

Most studies on the interaction between phytic acid/inositol phosphates and minerals reveal the existence of an inverse relationship between the absorption of these micronutrients and inositol phosphates, although there are substantial differences in individual behaviour of each mineral element [16].

The interaction of phytic acid with minerals and other nutrients is pH-dependent [30], since the degree of protonation of the phosphate groups is a function of pH [31]. The molecule works in a wide region of pH as a highly negatively-charged ion, so its presence in the diet has an adverse impact on the bioavailability of mineral monovalent, divalent and trivalent ions, such as Zn^{2+}, Fe^{2+} / Fe^{3+}, Ca^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+}[32-34]; these complexes are more soluble at low or acid pH and insoluble at high or basic pH [35].

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Another important aspect to be considered is that the interaction of phytic acid with minerals is due to its several phosphate groups, thereby, minerals may bind to one, two, or more phosphate groups of one, two or more phytic acid molecules [36]. Other studies have shown that the inhibitory effect of the absorption of InsP depends on the degree of phosphorylation of inositol, when it is high (5 or 6 phosphates) the absorption of Ca and Zn is significantly inhibited, however at lower levels of phosphorylation this effect is not observed [34].

The solubility of the complexes formed depends also on the InsP-mineral ratio; for instance, the solubility of the InsP-Ca complex, is extremely low in 1/8 ratios, but other ratios show higher solubility [37]. The complexes hexa-, penta-, tetra- and tri- Ca are insoluble, while complexes mono- and di- Ca are soluble [38]. Nevertheless, the absorption of Cafrom soluble complexes InsP-Ca is very low, because these complexes do not undergo passive transport in gut due to the high electric charge they have [39].

The inositol phosphates directly or indirectly interact with various minerals in the diet to reduce their bioavailability, in this context, the synergistic effect of the secondary cations (Ca^{2+}) has been widely demonstrated. Two cations may, when present at the same time, act together to increase the amount of insoluble precipitate in salt form, *i. e.* a mineral has a higher affinity for certain complex (InsP-mineral) which generates more insoluble salts[40]. For instance, the phosphate inositol bound to Ca shows higher affinity to Zn, which decreases its reabsorption [41].

1.1.3. Bioavailability of proteins

The degree of interaction between phytic acid (and its phosphate inositols) and depends on the protein, net charge, conformation and interactions with minerals at a given pH.At low pH, below the isoelectric point of proteins, phytic acid phosphate esters bind to the cationic group of basic amino acids, for example, arginine, histidine and lysine, may form InsP-protein complexes.

At a pH above the isoelectric point of proteins, since the charge of proteins as well as that of the phytic acid is negative, the interaction would be impossible, however, interaction occurs through the formation of complexes with divalent such as Ca^{2+} or Mg^{2+}. This binding takes place via the formation of ionized carboxyl groups and the deprotonated imidazole group of histidine, which requires a minimum concentration of these cations to maintain these complexes. At this pH, some binary complexes may exist because lisyl and arginyl residues of the proteins are still positively charged. At high pH the interaction between proteins and phytic acid decreases, arginyl and lysyl groups lose their charge, and therefore its ability to form binary complexes [36]; as well, they may form complexes such as protein-InsPand protein-mineral-InsP(Figure 4), which reduces their bioavailability.Such complexes may affect the protein structure, which may decrease the enzyme activity, function, solubility, absorption and protein digestibility [16, 36]. Particularly, the ability to inhibit proteolytic(pepsin, trypsin, chymotrypsin), amylolytic (amylase) and lipolytic (lipase) enzymes, is responsible of their anti-nutritional properties. This inhibition may be due to the nonspecific nature of InsP-protein interactions and the chelation of calcium ions, which are essential for the activi-

ty of trypsin and α-amylase [16]. Furthermore, phytic acid can also bind to starch, either directly via hydrogen bonds or indirectly through the proteins to which it is associated [42].

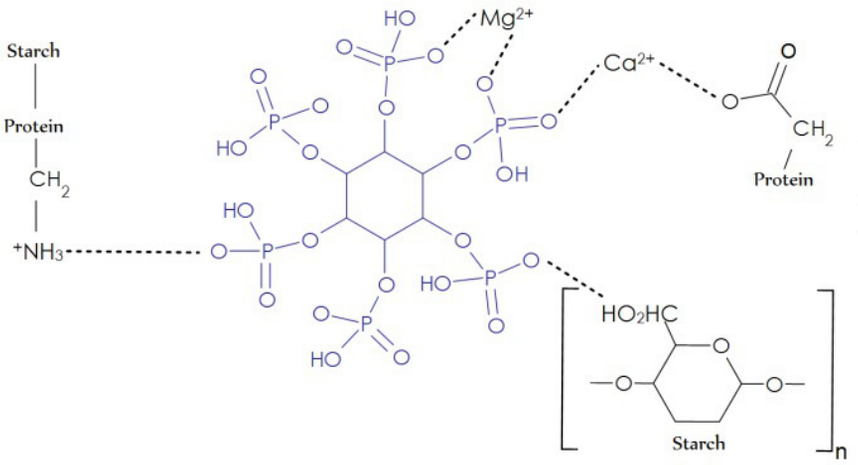

Figure 4. InsPinteractions with minerals, proteins and starch [36].

1.1.4. Pharmacological properties

In vivo and *in vitro* studies have shown that phytic acid ($InsP_6$)has prevention and therapeutic properties against cancer. Several mechanisms have been suggested to explain its anticarcinogenic effect:

a. Experiments have shown that this compound induces apoptosis in cancer cells, causes differentiation of malignant cells and its reversion to normal phenotype, and increases the activity of natural killer cells of the immune system. In addition, IP_3 and IP_4 compounds have an important role in cellular signal transduction, regulating functions, cell growth and differentiation [43].

b. A second way in which phytic acid reduces the risk of cancer, is by chelation of Fe^{3+} and the suppression of the formation of radicals (•OH), which also originates antioxidant properties. Fe^{3+} is an effective catalyst for many biological functions, in which this ion is reduced to Fe^{2+}. The oxidation of $Fe^{2+}$to Fe^{3+} leads to the formation of $O^{2-\bullet}$ that spontaneously generates O_2 and H_2O_2. The Fenton's reagent ($Fe^{2+} + H_2O_2$) quickly generates •OH, a highly-reactive oxyrradical which indiscriminately attacks most of the biomolecules. By blocking the redox cycle of Fe, which is necessary in many oxidation reactions, the lipid peroxidation and DNA damage are inhibited *[37 , 44].*

c. Zn is involved in DNA synthesis and cell proliferation as a cofactor for many enzymes like thymidine kinase. So, by binding to Zn^{2+}, the phytic acid indirectly reduces cell proliferation [45].

d. Phytic acid can reduce the starch digestibility and cause low absorption, so that starch remains available in the colon to be fermented by bacteria, producing short chain fatty acids, which have protective activity against cancer [46].

Phytic acid can retard the digestion and absorption of starch in several ways: by direct binding to the polysaccharide, by its binding to the α-amylase, or by chelation of Ca^{2+} needed for activation of α-amylase. Through these mechanisms a delay occurs in the glycemic response, therefore, due to lower blood glucose, insulin is required in less amount and this reduces the risk of diabetes [47].

Respect to prevention of kidney stones and treatment of hypercalciuria, experimental evidences demonstrate that di- and tri- inositol phosphates ($InsP_2$ and $InsP_3$), are effective to prevent the formation of hydroxyapatite crystals *in vitro*, which act as the core for the formation of some kidney stones [24].

At levels from 0.2 to 9% of phytic acid in diet, the plasmatic levels of cholesterol and triglycerides are significantly reduced [48]. This seems to be related with the capability of phytic acid to be bound to Zn, which reduces the serum levels of Zn and the Zn/Cu ratio, since high values of this ratio tend to increase the risk of cardiovascular diseases, for instance, hypercholesterolemia [42].

1.2. Saponins

Saponins (Figure 5) are a big group of glycosides which are known by their surfactant properties and are widely distributed in green plants [49] .The name 'saponin' derives from the Latin word *sapo* which means soap, due to their property of generating foam in agitated aqueous solutions [50]. These substances are amphiphilic glycosides, wherein the polar constituents are sugars (pentoses, hexoses or uronic acids) that are covalently linked to a nonpolar group, which consists of an aglycone, called sapogenin, which can be either steroidal or triterpenoid. This combination of polar and nonpolar components in their molecular structure explains their surfactant property in aqueous solutions [51].

As mentioned above, the saponins are secondary metabolites that can be classified into two groups based on the nature of the aglycone skeleton. The first group consists of steroidal saponins, which are present almost exclusively in monocotyledons angiosperms. The second group is composed of triterpenoidsaponins, which occur mainly in dicototyledonous flowering plants [52]. Steroidal saponins comprise a steroidal aglycone, a spirostane skeleton of 27 carbons (C_{27}), which generally comprises a six-ring structure. In some cases, the hydroxyl group at position 26 is used to form a glycosidic bond, so that the structure of the aglycone becomes a pentacyclic structure; this structure is known as furostano skeleton. The triterpenoidsaponins have an aglycone with a backbone of 30 carbons (C_{30}), which form a pentacyclic structure (Figure 5).

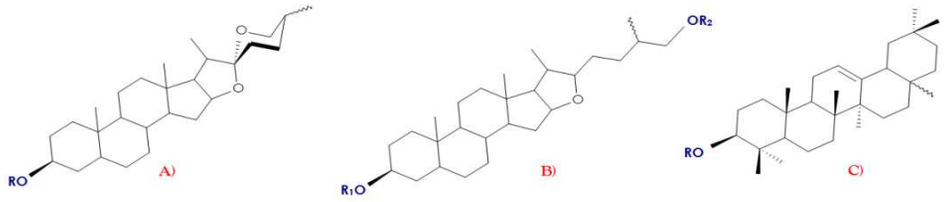

Figure 5. Skeletons ofaglycone: (A) steroidal spirostane, (B) steroidal furostane (C) triterpenoid. R = sugar residue.

It has been identified that soy contains saponins with triterpenoid-type aglycones, this kind of aglycones are subdivided into five major groups; soysapogenol A, B, C, D and E (Figure 6), and their glycosides are correspondingly called as saponins of group A, group B, and so on[53, 54].From this classification, four aglycones (soysapogenol A, B, C and E) [55]were isolated after hydrolisis of soy saponins, specifically five saponins were identified 5 with two distinct types of aglycones: soysapogenin I (the main component), soysapogenins II and III, which contain soysapogenol B, and soysaponins A1, A2 and A3, which contain soysapogenol A[55].The saponins containing soyasapogenol C and E have not been found in soybeans, so these aglycones could be formed as a product during the hydrolysis of saponins[56].Another study reported the isolation and characterization of soysaponin IV. The type of sugars attached to the aglycones found in soybeans have been identified as rhamnose, galactose, glucose, arabinose, xylose and glucuronic acid [55].

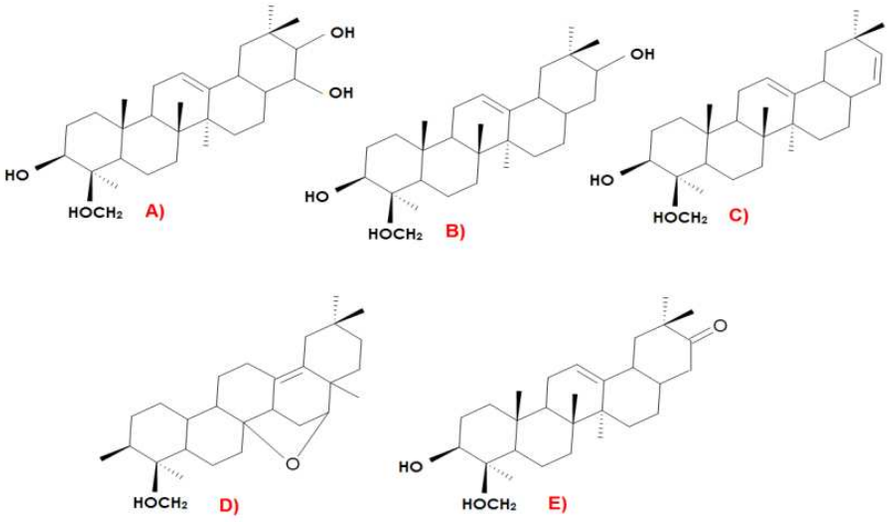

Figure 6. Groups of soyasapogenols *(Oakenfull, 1981).*

The total content of saponins in the hypocotyl fraction of soybeans, where acetyl-soyasaponins A1 and A4, mainly, are syntesized, is approximately 0.62 to 6.16%[57]. Other works have reported 5-6% saponin content in soybeans [58]. Lower values, approximately 0.6%, have been reported as well [59].

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1.2.1. Synthesis and Function

The capability to synthesize saponins is widespread among plants belonging to the *Magnoliophyta* division, which includes both dicotyledons and monocotyledons. However, most of saponin-producing species are within dicotyledonous plants. The biological function of saponins is not completely understood. They are usually considered as a part of the defense system of the plant, due to their antimicrobial, fungicide, allelopathic, insecticide and molluscicide activities [60]. The synthesized saponins are accumulated during the regular growth of plants. Nonetheless, their accumulation is influenced by several environmental factors such as bioavailability of nutrients and water, solar radiation or a combination of them[61]. Some studies on soy have shown a variation in the content of saponins in soybeans with different degrees of maturity, however, the nature of this variation is not sufficient to influence on the saponindistribution in different varieties [57]. Little is known about the enzymes and biochemical pathways involved in the biosynthesis of saponins in plants [54]. However, two key aspects have been suggested for biosynthesis: the first one is the cyclization of the 2,3-oxidoscualene thought the isoprenoid pathway, which is a starting point for the biosynthesis of the sapogenin, and the second one is the glycosylation of sapogenins.

1.2.2. Membranolytic activity

Saponins have the ability to cleavege the erythrocytes. This hemolytic property is generally attributed to the interaction between saponins and sterols in the erythrocyte membrane. As a result, the membrane is broken, which causes an increase in its permeability and the consequent loss of hemoglobin. It has been investigated the effect of saponins in the membrane structure through human erythrocyte hemolysis[5, 62]. The results indicated that the fracture in the erythrocyte membrane was not closed again, so that the damage in the lipid bilayer is irreversible. However, this toxic property is difficult to occur *in vivo*, since there is evidence that no complications are detected when saponins are ingested orally, which reduces his hemolytic capability to *in vitro* studies [63]. The saponins have little anti-nutritional activity, given no damage is produced in humans when they are consumed in the amounts regularly found after food processing. However, high concentrations of saponins are also capable of breaking the membrane of other cells such as those of the intestinal mucosa, which modifies the cell membrane permeability, and then affects the active transport and the absorption of nutrients[45].

This ability to affect the cell membrane, depends on the structural characteristics of the saponins, *i. e.* the structure of the aglycone, the number of sugars in the side chains and the side chains length [64]. In Figure 7, the interaction of saponins with cell membranes is schematically shown.

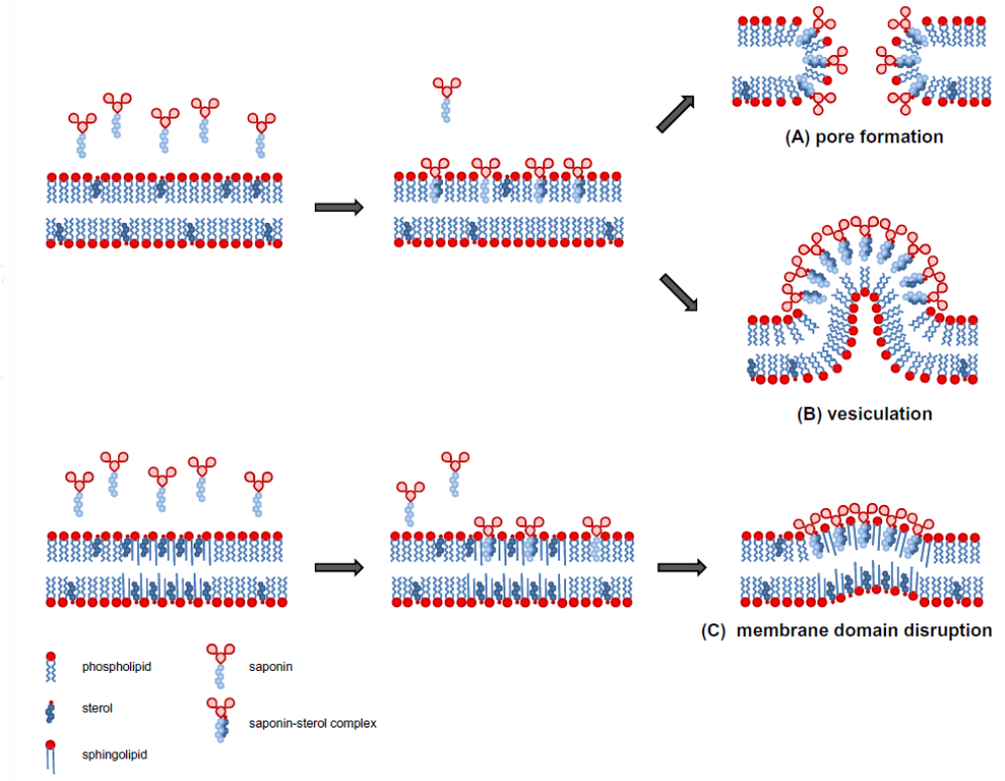

Figure 7. Schematic models of the molecular mechanisms of saponin activities towards membranes [65]Saponins integrate with their hydrophobic part (sapogenin) into the membrane. Within the membrane they form complexes with sterols, which subsequently, driven by interaction of their extra-membranous orientated saccharide residues, accumulate into plaques. Sterical interference of these saccharide moieties causes membrane curvature subsequently leading to (A) pore formation in the membrane [66] or (B) hemitubular protuberances resulting in sterol extraction via vesiculation[67]. Alternatively, after membrane integration saponins may migrate towards sphingolipid/sterol enriched membrane domains (C) prior to complex formation with the incorporated sterols, thereby interfering with specific domain functionalities [68]. Similarly to (B), accumulation of saponins in confined membrane domains has further been suggested to cause deconstructive membrane curvature in a dose-dependent manner.

1.2.3. Pharmacological properties

Many studies highlight the pharmaceutical properties of the soybean saponins, among which the anti-carcinogenic activity is mentioned, given by the membranolyticactivity these molecules have shown in human cells of colon carcinoma[69, 70]. Other studies have demonstrated hypocholesterolemic activity due to depletion of body cholesterol by preventing its reabsorption, thus increasing its excretion. Soluble fibers in legumes are known to increase the viscosity of gastric and intestinal contents, and may be one of the factors responsible for the lowering of cholesterol levels [71, 72]. Studies on health benefits of saponins suggest their hepatoprotective activity, but these studies are limited to cell culture and few animal studies. Studies on rats have shown soybean saponin to have an anabolic effect on bone components, suggesting its role as a nutritional factor in the prevention of osteoporosis [73]. Another activity that has been reported is anti-mutagenicity in breast cells [74].

1.3. Inhibitors of trypsin

Protease inhibitors are proteins widely distributed in the plant kingdom, have the ability to inhibit the proteolytic activity of digestive enzymes such as serine-proteases (trypsin and chymotrypsin) which are characteristic of the gastrointestinal tract of animals, though also may inhibit endogenous proteases and enzymes of bacteria, fungi and insects. These serine-protease inhibitors are proteins that form very stable complexes with digestive enzymes, which preventtheir catalytic activity [75].

Protease inhibitors have been classified into several families based on homology in the sequence of amino acids in the inhibitory sites. The molecular structure of the inhibitor affects both the force and the specificity of the inhibitor. The two main families of protease inhibitors found in legumes are the Kunitz inhibitor and the Bowman-Birk inhibitor, so named after its isolation [2, 51]. In the latter case, the characterization was carried out by *Birk, [76]*, so this name was added.

Both types of proteases are found in soybeans (*Glycine max*); in other legume seeds, such as beans (*Phaseolus vulgaris*) and lentil (*Lens culinaris*), protease inhibitors have been characterized as members of the Bowman-Birk family. Both inhibitors are water soluble proteins (albumin) and constitute from 0.2 to 2% of total soluble protein of legumes [75, 77], particularly soybeans havereported 50 trypsin inhibitor units / mg of dry sample [78].

1.3.1. Kunitztype inhibitor

The first protease inhibitor to be isolated and characterized was the Kunitz inhibitor. It has a MW between 18 and 24 kDa and contains between 170 and 200 amino acid residues. These inhibitors have one head, i. e., one molecule of inhibitor inactivates one molecule of trypsin. It is a competitive inhibitor, binds to the active sites of trypsin in the same way the substrate of the enzyme does, resulting in the hydrolysis of peptide bonds between amino acids of the reactive site of the inhibitor or the substrate (Figure 8).

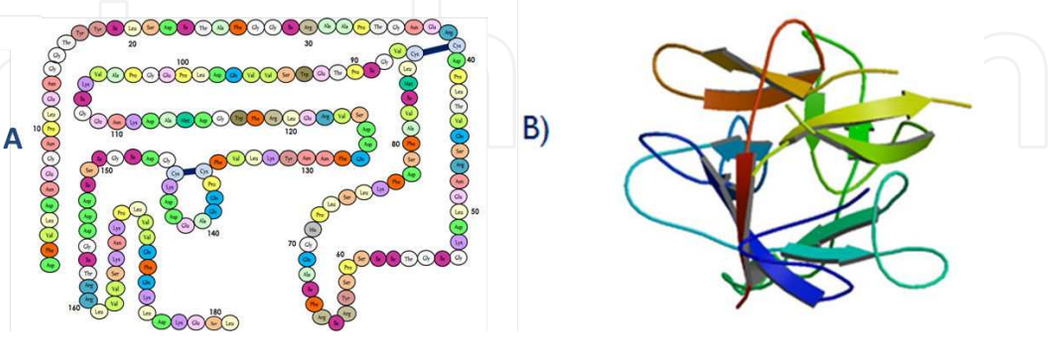

Figure 8. A) Primary structure of the Kunitz inhibitor from soybean [79]. Disulphide bonds are shown in black, B)Tridimentional structure Kunitz inhibitor from soybean [79].

Inhibitors differ from the substrate protein in the reactive site residues, which are linked via disulphide bonds. After hydrolysis, the modified inhibitor maintains the same conforma-

tion, due to the disulphide bonds. This generates a stable enzyme-inhibitor complex. This type of inhibitors are generally absent in seeds of *Phaseolus*, *Pisum*, *Vigna unguiculata* and *Glycine max* [*80*].

1.3.2. Bowman-Birk type inhibitor

These inhibitors are low molecular weight polypeptides (7 to 9 kDa) containing 60 to 85 amino acid residues (Figure 9). They have several disulphide bonds which make them stable to heat, acids and bases. These inhibitors have two heads (two separate sites of inhibition) and are competitive inhibitors. They can simultaneously and independently inhibit two enzymes, thus, there are trypsin/trypsin are trypsin/chymotrypsin inhibitors [74,77].

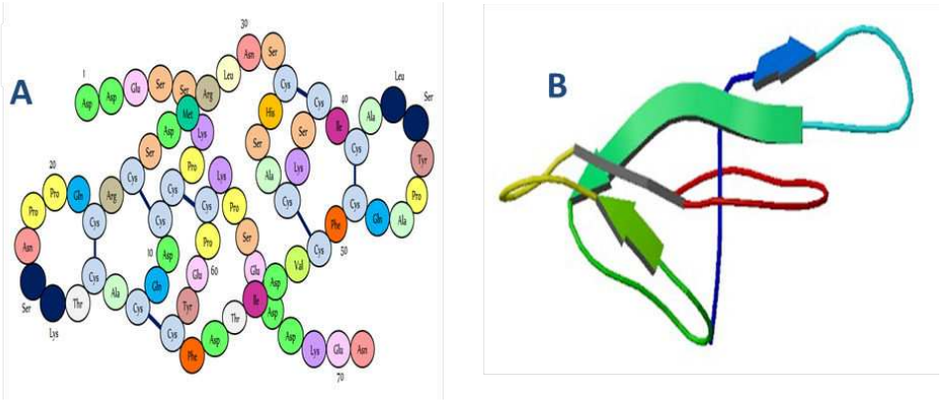

Figure 9. A) Primary structure of Bowman-Birk type inhibitor from soybean*(Odani y Ikenaka, 1973). Disulphide bondsand active sites for trypsin (Lys16-Ser17) and chymotrypsin (Leu44-Ser45) are shown in black B)* Tridimentional structure Bowman-Birk inhibitor from soybean [81]

An example of this type of inhibitor is the Bowman-Birk inhibitor from soybeans, which is constituted by a polypeptide chain of 71 amino acids, containing seven disulphide bonds. It has a MW of 8 kDa and is called dual head inhibitor because it has independent binding sites for trypsin and chymotrypsin, so that the active site for trypsin is Lys16-Ser17, whereas for chymotrypsin is Leu44-Ser45 [82, 83].

1.3.3. Synthesis and Function

Protease inhibitors have a regulatory function; they are involved the in proteolytic self-regulation process of the protein deposited in the protein bodies before and during the seed germination by inhibiting endogenous proteases. They also participate as protective agents against insects and microorganisms[76].

1.3.4. Anti-nutritional properties

Protease inhibitors ingested within legumes have adverse effects in animals. First, these compounds form inactive complexes with trypsin/chymotrypsin, so that the levels of these free digestive enzymes are reduced, thus making difficult proteolysis and amino acid ab-

sorption. In addition, these enzyme-inhibitor complexes, which are rich in sulfur amino acids, are excreted.

Finally, these inhibitors cause hypertrophy/hyperplasia of the pancreas due to chronic hypersecretion of pancreatic enzymes [trypsin and chymotrypsin), which leads to deviation of the sulfur amino acids that were used to synthesize tissue proteins to the synthesis of these enzymes [84]. All this derives in reduction of the amount of essential amino acids, which inhibits animal growth and exacerbates an already critical situation with respect to the protein of legumes which is deficient in sulfur amino acids [3, 84-87].

The mechanism by which inhibitors of proteases stimulate pancreatic secretion is not entirely clear. There is a theory about this secretion would be regulated by a negative feedback mechanism, so that, when the content of trypsin/chymotrypsin in duodenum is reduced below a certain level, the endocrine cells of the duodenal mucosa release the hormone cholecystokinin, prompting the pancreas to synthesize more serine-proteases (Figure 20). The reduced levels of trypsin and chymotrypsin are produced when the protease inhibitors ingested reach the duodenum and bind to these digestive enzymes by forming complexes. Although this does not seem to be the only mechanism by which the pancreatic secretion is activated.

Recent studies have demonstrated that both states of protease inhibitors, free and enzyme-inhibitor complexes, bind to the duodenal mucosa and stimulate the release of cholecystokinin, thus increasing the pancreatic secretion of serine proteases [84, 88]. The action of the trypsin inhibitors on the human organism is not totally understood, since human trypsin has two forms: cationic, which is the main component of pancreatic juice and is weakly inhibited; and anionic, comprising about 10 to 20% of the total trypsin, which is completely inhibited [82, 84].

1.3.5. Pharmacological properties

Since the Bowman-Birk type inhibitors are proteins with a high amount of cysteine , these inhibitors make an important contribution to the content of sulfur amino acids, thus increasing the nutritional value of legumes[85, 89]. The Bowman-Birk inhibitor from soybeans as well as their counterparts present in other legumes, are involved in the prevention and treatment of cancer (colon, breast, liver, lung, prostate, etc.) by inhibiting chymotrypsin. One mechanism through which these compounds can prevent carcinogenesis is by reducing the protein digestibility and the bioavailability of amino acids such as leucine, phenylalanine or tyrosine, which are necessary for the development of cancer cells [90-92].

1.4. Isoflavones

Isoflavones are widely distributed in the plant kingdom, mainly in plants of the legume family, being soybeans the source with the highest content of these components [93]. Isoflavones are oxygen heterocycles containing a 3-phenylchroman skeleton that is hydroxylated at 40 and 7 positions *(Figura 10)* [94]. Based on the substitution pattern on carbons and 6, three aglycon forms of isoflavones commonly found in soybeans are daidzein, genistein, and glycitein. These

three isoflavones can also exist in conjugated forms with glucose (daidzin, geinstin, glycitin), malonylglucose (malonyldaidzin, malonylgeinstin, malonylglycitin), and acetylglucose (acetyldaidzin, acetylgeinstin, acetylglycitin) units. Thus 12 free and conjugated forms of isoflavones have been isolated from different soybean samples (Table 1) [95].

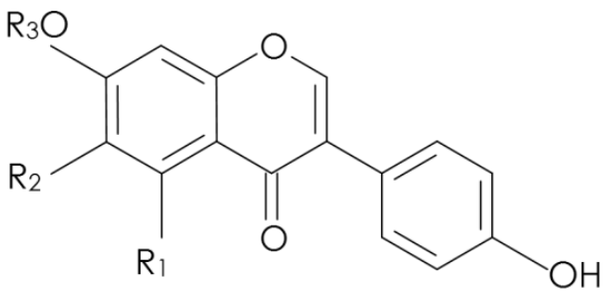

Figure 10. Chemical structureof an isoflavone [95].

Name	R_1	R_2	R_3
Daidzein	H	H	H
Glycitein	H	OCH_3	H
Genistein	OH	H	H
Daidzin	H	H	Glu
Glycitin	H	OCH_3	Glu
Genistin	OH	H	Glu
Acetyldaidzin	H	H	Glu-$COCH_3$
Acetylglycitin	H	OCH_3	Glu-$COCH_3$
Acetylgenistin	OH	H	Glu-$COCH_3$
Malonyldaidzin	H	H	Glu-$COCH_2COOH$
Malonylglycitin	H	OCH_3	Glu-$COCH_2COOH$

Table 1. Chemical structures of 12 isoflavones isolated from soybeans [95].

1.4.1. Synthesis and Function

The variation of the concentration of isoflavones in soybeans is mainly due to the soybean variety, environment, location and growing conditions, such as year, area and temperature, post-harvest storage and the methodology used to determine this concentration [95]. The content of isoflavones in soybeans ranks 1.2 to 2.4 mg of total isoflavones per gram of sample [96], distributed in different concentrations in the tissues of the seed, being higher in the embryo than in the endosperm [97].

In plants, these compounds play several roles, such as protection against UV light and phytopathogens, signal transduction during nodulation, attraction of pollinator animals and defense against insects and herbivores[98].

1.4.2. Pharmacological properties

The enriched extracts of isoflavones have been evaluated for prevention of a wide range of health problems associated with cardiovascular diseases, osteoporosis and breast cancer, prostate and colon [99]. Furthermore, soy isoflavones have a structure very similar to a phenolic estrogen known as phytoestrogen, so that these compounds have been used as a natural alternative for postmenopausal therapy [100].

1.5. Lectins

In soybeans, a class of proteins called lectins or phytohemagglutinins is present. These compounds can be defined as proteins or glycoproteins of non-immune origin, which can reversibly bind to specific sugar segments through hydrogen bonds and Van Der Waals interactions, with one or more binding sites per subunit [101]. Lectins are tetrameric proteins composed of two different types of subunits: E-type subunit (MW = 34 kDa) and L-type subunit (32 kDa). The first one has the characteristic of binding to erythrocytes, while the second one to lymphocytes[102].Therefore, it is possible to find 5 combinations of these four subunits, i. e. 5 isoforms, as follows: E_4, E_3L, E_2L_2, EL_3, and L_4. Soybean seeds show hemagglutinating activity at 2400 mg per mg of dry sample [78].

The name lectin [from Latin legere, which means to choose or to select), was adopted by Boyd for many years to emphasize the caability of some lectins to bind specifically to cells of the ABO blood groups [103]. Currently the name lectinis preferred over the haemagglutinin one and is widely used to denote all vegetable proteins that possess at least one non-catalytic domain, which binds reversibly to a specific mono- or oligosaccharide [104].

According to the overall structure of the plant lectins, these are subdivided into four main classes: Merolectins which are proteins having a single carbohydrate-binding domain; Hololectins, comprising all lectins having di- or multivalent carbohydrate-binding sites; Chimerolectins, proteins consisting of one or more carbohydrate-binding domain(s) plus an additional catalytic or another biological activity dependent on a distinct domain other than the carbohydrate- binding site; and Superlectins which also possess at least two carbohydrate-binding domains but differ from the hololectins because their sites are able to recognise structurally unrelated sugars [105].

Lectins can be divided according to the monosaccharide for which they show the highest affinity: D-mannose/D-glucose, D-galactose/N-acetyl-D-galactosamine, L-fucose and N-acetylglucosamineacid [106]. Thus depending on the specificity toward a given monosaccharide the lectin will selectively bind to one of these above sugars which are typical constituents of eukaryotic cell surfaces [101].

1.5.1. Synthesis and Function

The wide distribution of lectins in all tissues of plants and their ubiquitous presence in the plant kingdom suggest important roles for these proteins. One possible physiological function that has emerged is the defensive role of these carbohydrate-binding proteins against phytopathogenic microorganisms, phytophagous insects and plant-eating animals [102, 107]. Indeed it has been shown that plant lectins possess cytotoxic, fungitoxic, anti-insect and anti-nematode properties either in vitro or in vivo and are toxic to higher animals[63, 81, 104]. One of the most important features of plant lectins, compatible with the proposed defensive function, is the remarkably high resistance to proteolysis and stability over a large range of pH, even when they are out of their natural environment [103].

1.5.2. Anti-nutritional properties

Some of these were found to be toxic or antinutritional for man and animals. In general, nausea, bloating, vomiting and diarrhoea characterize the oral acute toxicity of lectins on humans exposed to them.

In experimental animals fed on diets containing plant lectins the evident symptoms are loss of appetite, decreased body weight and eventually death [84, 108].

As most lectins are not degraded during their passage through the digestive tract they are able to bind the epithelial cells which express carbohydrate moieties recognised by them. This event is undoubtedly the second one in importance for determining the toxicity of orally fed lectins. Indeed, lectins which are not bound by the mucosa usually induce little or no harmful antinutritive effect for the consumers [88]. Once bound to the digestive tract, the lectin can cause dramatic changes in the cellular morphology and metabolism of the stomach and/ or small intestine and activate a cascade of signals which alters the intermediary metabolism. Thus, lectins may induce changes in some, or all, of the digestive, absorptive, protective or secretory functions of the whole digestive system and affect cellular proliferation and turnover. In 1960, Jaffe´ suggested that the toxic effects of ingested lectins were due to their ability to combine with specific receptor sites of the cells lining the small intestine and to cause a non-specific interference with absorption and nutrient utilisation [50].

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