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Review article

Section: Food Quality and Functionality

## Non-Nutritive Compounds in *Fabaceae* Family Seeds and the Improvement of Their Nutritional Quality by Traditional Processing – a Review

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The consumption of seeds of the *Fabaceae* family is distributed worldwide mainly due to their high content of proteins, carbohydrates, dietary fiber and polyunsaturated fatty acids, jointly with vitamins and minerals. However, they contain several non-nutritive compounds (NNCs) that can exert adverse or beneficial actions upon ingestion depending on their chemical structure, concentration, time of exposure and their interaction with other dietary components. In this review, we present the representative legume NNCs, their chemical nature and their adverse and beneficial biological actions. Moreover, we summarized updated findings on the effect of different traditional processing, bearing in mind that legumes are mainly consumed in the household milieu, on the concentration of legume NNCs. The results of the *in-vivo* studies prove that the reduction/elimination of legume NNCs improves nutritional quality and the fate of improvements depends on many parameters such as botanical source, chemical composition, content, type of processing and operational conditions used, among others. Together, this review can provide a comprehensive perspective for further elucidating the roles of plant lectins that may target programmed cell death pathways. This review may, in turn, ultimately help to consumers for whom legumes are part of a vegetarian diet or are consumed as staple food which must take into consideration the improvement of legume nutritive quality by traditional processing.

### INTRODUCTION

The seeds of the *Fabaceae* family have been of particular interest to nutritionists as a rich source of protein, fat, carbohydrates, vitamins and microelements, for which they have been recognized as nutritive food. Some examples are the seeds of *Lupinus luteus* which contain about 50% protein, the seeds of *Lupinus mutabilis* and *Glycine max* which contain over 20% of fat rich in unsaturated fatty acids, and *Lens culinaris* containing 55% of carbohydrates. Unfortunately, the seeds of different species belonging to this botanical family also contain non-nutritive compounds (NNCs) that decrease nutrient bioavailability affecting legume nutritional quality. In addition, some NNCs exert toxic effects in living organisms when they are consumed at high doses, which limit in some cases legume utilization in human nutrition. Some NNCs are secondary plant metabolites, which are accumulated during seed development in response to biotic and abiotic defense mechanisms against insect plagues or adverse environmental conditions. These compounds can be classified as NNCs of protein origin such as trypsin inhibitors and hemagglutinins that are thermolabile, and NNCs of non-protein origin like phytic acid, raffinose family oligosaccharides (RFOs or

$\alpha$ -galactosides), tannins, alkaloids, vicine, convicine and saponins, which are heat stable but can be reduced by dehulling, soaking and cooking, germination and fermentation. A very interesting fact is that particular genera are characterized by specific NNCs. In this sense, *Lupinus* contains alkaloids and RFOs; *Glycine* and *Pisum* are rich in protease inhibitors and hemagglutinin; genus *Lens* contains trypsin inhibitors, condensed tannins and phytates; *Phaseolus* is characterized by the flatulent RFOs, trypsin inhibitors, hemagglutinin and tannins; *Cicer* contains reasonable levels of trypsin inhibitors, hemagglutinin, tannins and saponins; while phytic acid, saponins and tannins predominate in genus *Vigna*.

Legume NNCs attracted the attention of many researchers during the 80's and 90's. However, from the early 2000's several studies have shown that many of these compounds can be also considered as pronutrients with positive health effects contributing to the prevention of diseases. The balance between detrimental and beneficial effects of these compounds depends on many factors, such as plant origin, chemical structure, concentration and processing conditions. Although some compounds may exert beneficial effects they are still considered by nutritionists as a disadvantageous from a nutritional point of view, mainly for people consuming vegetarian diets or consuming legumes as staple food. Hence, their total or partial removal is essential to improve the le-

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gume nutritional quality and their utilization. In this sense, this review provides updated information on several methods of conventional processing including dehulling, germination, soaking, cooking, fermentation, extrusion, solvent extraction, dry-heating and enzymatic treatments. Other outstanding possibilities as plant breeding, agronomic practices, genetic engineering or even biotechnology application, which have been successfully used to reduce NNCs present in crops are not considered here.

## NON-NUTRITIONAL COMPOUNDS OF PROTEIN ORIGIN

### Protease inhibitors

Protease inhibitors (PI) are found in most plant organisms. They are widely distributed among different botanical families and are particularly abundant (1–10% of total proteins) in storage organs like seeds and tubers [Birk, 2003]. PI have been isolated and characterized from many legume seeds, e.g. soybean, tepary bean, navy bean, faba bean, cowpea, bambara groundnut and pigeon pea [Benjakul *et al.*, 1999; Gupta *et al.*, 2000; Campos *et al.*, 2004; Sammour, 2005; Guillamon *et al.*, 2008].

The PI isolated from different species differ by molecular weight, amino acid composition and location of the active site. In most cases, PI occurring in legumes consist of two types, the Bowman-Birk (BBI; 8 kDa) and the Kunitz trypsin family inhibitors (20–24 kDa) [Wati *et al.*, 2009]. The first one is a small serine proteinase inhibitor containing 71 amino acids, rich in disulfide bonds, which has two separate protease inhibitory sites capable of inhibiting trypsin and chymotrypsin enzymes [Winiarska-Mieczan, 2007]. Proteins from the Kunitz family contain 170 to 200 amino acid residues and one or two intra-chain disulfide bonds. Although the accurate mechanism of PI action is not well-known, it is thought to consist in the formation of non-active complexes with these proteolytic enzymes as well as elastase cathepsin, among others. PI isoforms vary with legume species and varieties [Guillamon *et al.*, 2008]. High consumption of PI leads to a reduced availability of assimilable proteins conducting in a long term to reduced growth and pancreas hypertrophy.

On the other hand, protease inhibitors have shown a therapeutic effect in cancer and inflammatory diseases such as multiple sclerosis and cancer [Safavi & Rostami, 2012]. Several BBI mechanisms have been reported in cancer prevention. BBI suppresses the activity of membrane-type serine protease 1, which plays a significant role in cancer invasion and metastasis [Yamasaki *et al.*, 2003], and proteasome function resulting in suppression of breast cancer cell growth [Chen *et al.*, 2005]. In addition, BBI enhances apoptosis and lysosome membrane permeabilization in MCF-7 breast cancer cells [Joanitti *et al.*, 2010].

Research studies have shown that the PI content ranges from negligible amounts in *Lupinus* spp. (0.5–0.9 TIU/mg) to very high in *Glycine max.* (43–84 TIU/mg), *Phaseolus vulgaris* (21–25 TIU/mg), *Lathyrus* spp. (19–30 TIU/mg) and varies among legume species and cultivars [Bastianelli *et al.*, 1998; Duc *et al.*, 1999; Filippetti *et al.*, 1999; Vidal-Valverde *et al.*, 2003; Gabriel *et al.*, 2008; Guillamon *et al.*, 2008; Wang *et al.*,

2009; Vasconcelos *et al.*, 2010]. The levels of PI activity of different legume species and cultivars are collected in Table 1.

Many studies have been conducted to find an effective approach to remove the PI due to their implications on reducing the protein availability of legumes. Thermal treatments are the most used for the removal of these heat-sensitive compounds. Vidal-Valverde *et al.* [1994] showed that cooking of presoaked lentil seeds rich in PI brought about the total removal of PI activity. In these studies it was shown that soaking was the least effective process. This finding agrees with the study carried out in pea seeds [Wang *et al.*, 2008a]. Embaby [2010] showed that the dehulling and soaking processes of bitter and sweet lupine seeds for 96 and 24 hours, respectively, caused a significant increase of trypsin inhibitor activity, whereas cooking led to its inactivation. The cooking and autoclaving were the most effective processes for the elimination of PI activity from both lupines [Embaby, 2010].

PI can also be extracted by using solvents at alkaline pH, aqueous salt solutions at different pH [Tan *et al.*, 1982] or with water [Page *et al.*, 2000; Wati *et al.*, 2009]. Each method has its own advantages and disadvantages with different target protein components. In this sense, Wati *et al.* [2009] reported that extraction with 0.02 mol/L NaOH led to the highest recovery of PI from the navy bean and red kidney bean, while water was the best extractant for the adzuki bean. Although cooking is a very simple method for the reduction of PI activity, this process may lead to the elimination of other important compounds like polyphenols. Alonso *et al.* [1998] observed that extrusion of pea seeds at 148°C, 25% moisture and 100 rpm was the most effective for reduction of condensed tannins, trypsin, chymotrypsin, amylase inhibitors and hemagglutinating activity reduction without modification of protein content, which can occur with dehulling, soaking and germination treatments. In addition, PI, chymotrypsin inhibitors and hemagglutinating activities were more readily inactivated by the extrusion treatment than the chymotrypsin inhibitory activity.

### Hemagglutinins

Hemagglutinins (HG), also called lectins, constitute an ubiquitous class of proteins which are widely distributed in the plant kingdom. They play an important physiological role in humans, animals and plants [van Rhijn *et al.*, 2001; Duranti, 2006]. According to Barampama *et al.* [1993], the content of HG in four varieties of dry beans (*Phaseolus vulgaris* L.) was  $2.15 \times 10^{-3}$  HG units/mg, and its distribution seems not to be equal in seed parts since they found HG activity only in cotyledons and it was about 10% of that of *Glycine max* and *Pisum sativum*, and 2% in that of *Vicia faba*. In *Val-laris sinensis* and *Lupinus* seeds, HG activity is negligible.

HG belongs to the carbohydrate-binding proteins of non-immune origin, which agglutinate cells or precipitate polysaccharides and glycoconjugates. They have at least one non-catalytic domain that binds reversibly to specific monosaccharides or oligosaccharides. HG were, long ago, the subject of interest in many fields, such as hematological diagnostics, biological chemotaxonomy and nutrition sciences for the sake of physiological function in plant and biological properties [Wyciór *et al.*, 2008; Vandenborre *et al.*, 2011; Sze & Tzi, 2011]. Al-

TABLE 1. The content of antinutritional factors in different legume species.

Plant species	Antinutritional factors						
	TIA (TIUmg <sup>-1</sup> )	HG (HU×10 <sup>-3</sup> /mg)	PA (mg/g)	RFOs (mg/g)	Tannins (mg/g)	QA (% in dm)	Saponin (mg/g)
<i>Cicer arietinum</i>	15–19 <sup>1*</sup> 10.3–12.7 <sup>7</sup>	-	5.4–12.3 <sup>12</sup>	2.0–7.6 <sup>17,18</sup>	-	-	2.3 <sup>17,18</sup>
<i>Glycine max</i>	43–84 <sup>1</sup>	-	6.2–20.5 <sup>11</sup> 32.4–41.3 <sup>12</sup>	8.2 <sup>19</sup>	0.45 <sup>21</sup>	-	6.5 <sup>17</sup> 5.8 <sup>22</sup>
<i>Lens culinaris</i>	3–8 <sup>1</sup> 1.9–2.8 <sup>2</sup>	-	6.2–8.8 <sup>2</sup>	1.8–7.5 <sup>18</sup>	3.4–6.1 <sup>2</sup>	-	1.11 <sup>7,18</sup>
<i>Lupinus albus</i>	0.9 <sup>1</sup>	-	-	8.5–9.3 <sup>19</sup>	-	0.03–2.0 <sup>20</sup>	-
<i>L. angustifolius</i>	0.8 <sup>1</sup>	-	-	7.79–8.6 <sup>19</sup>	-	0.01–1.3 <sup>20</sup>	0.27–0.48 <sup>23</sup>
<i>L. luteus</i>	0.5 <sup>1</sup>	-	-	11.4–16.0 <sup>19</sup>	-	0.01–0.8 <sup>20</sup>	0.06–0.07 <sup>23</sup>
<i>Phaseolus vulgaris</i>	21–25 <sup>1</sup>	2.15 <sup>9</sup>	16.50 <sup>9</sup> 2.9–17.8 <sup>13</sup>	2.6 <sup>9</sup> 0.4–8 <sup>17,18</sup>	14.99 <sup>9</sup> 0–38.5 <sup>14</sup>	-	2.3–3.5 <sup>17,18</sup> 2.9–3.2 <sup>22</sup>
<i>Pisum sativum</i>	6–15 <sup>1</sup> 1–14.6 <sup>3</sup> 1.9–6.8 <sup>4</sup>	-	1.3–10.2 <sup>3</sup>	2.3–9.6 <sup>17</sup> 3.6–10 <sup>3</sup>	0.04–7.4 <sup>3</sup>	-	1.11 <sup>7,18</sup> 0.3–1 <sup>3</sup>
<i>Vicia faba</i>	5–10 <sup>1</sup> 0.3–5.3 <sup>5</sup> 0.8–3.6 <sup>6</sup>	-	3.8–13.4 <sup>5</sup>	1.4–6.2 <sup>5</sup> 1.0–4.5 <sup>17,18</sup> 1.0 <sup>19</sup>	0.1–10.4 <sup>5</sup> 2.1–3.2 <sup>15</sup> 20 <sup>21</sup>	-	0.11 <sup>7,18</sup>
<i>Vigna anguiculata</i>	12–16.6 <sup>8</sup>	-	-	-	0.3–6.9 <sup>16</sup>	-	-
<i>Vigna radiata</i>	-	-	10.2–14.8 <sup>12</sup>	-	-	-	-
<i>Cajanus cajan</i>	-	-	9.9–16.4 <sup>10</sup> 6.8–14.9 <sup>12</sup>	-	-	-	-
<i>Lathyrus</i> spp.	19–30 <sup>1</sup>	-	-	-	-	-	-
<i>Medicago sativa</i>	-	-	-	3.5 <sup>19</sup>	-	-	-
<i>Trifolium repens</i>	-	-	-	6.7 <sup>19</sup>	-	-	-
<i>Ornithopus sativus</i>	-	-	-	3.6 <sup>19</sup>	-	-	-

TIA – trypsin inhibitors activity; HG - hemagglutinins; PA - phytic acid; RFOs - raffinose family oligosaccharides QA - quinolizidine alkaloids;

\*References: <sup>1</sup>Guillamon et al. [2008]; <sup>2</sup>Wang et al. [2009]; <sup>3</sup>Bastianelli et al. [1998]; <sup>4</sup>Gabriel et al. [2008]; <sup>5</sup>Duc et al. [1999]; <sup>6</sup>Filippetti et al. [1999]; <sup>7</sup>Singh & Jambunatham [1981]; <sup>8</sup>Vasconcelos et al. [2010]; <sup>9</sup>Barampama et al. [1993]; <sup>10</sup>Singh [1999]; <sup>11</sup>Saghai Maroof et al. [2009]; <sup>12</sup>Chitra et al. [1995]; <sup>13</sup>Blair et al. [2009]; <sup>14</sup>Caldas & Blair [2009]; <sup>15</sup>Avola et al. [2009]; <sup>16</sup>Plahar et al. [1997]; <sup>17</sup>Kadlec et al. [2001]; <sup>18</sup>Kozłowska et al. [2001]; <sup>19</sup>Muzquiz et al. [1999]; <sup>20</sup>Gulewicz [1988]; <sup>21</sup>Reddy et al. [1985]; <sup>22</sup>Gurfinkel & Rao [2002]; <sup>23</sup>Muzquiz et al. [1993].

though the mechanism of unbeneficial action of lectins on animal organisms is still not well known, it is mostly recognized that their negative physiological effects are due to the ability to bind glycoproteins on the surface of microvilli lining of the small intestine, thereby interfering with the breakdown and absorption of nutrients. On the other hand, it is suggested that binding the lectins with intestine cell wall proteins causes the weakening of the immune response. Systemically, lectins can disrupt lipid, carbohydrate and protein metabolism, promote enlargement and/or atrophy of key organs and tissues and alter the hormonal and immunological status. At high intakes, lectins can threaten the growth and health of animals [Liener, 1997; Vasconcelos & Oliveira, 2004].

On the other hand, bean extracts enriched in lectins and lectin-like proteins are also finding a growing use as active ingredients of “weight-blockers” in dietetic preparations for obesity treatment [Pusztai et al., 1998]. The preparations are known to contain high levels of lectin-like proteins that inhibit  $\alpha$ -amylase, hindering digestion of complex carbohydrates, thereby promoting weight loss [Chokshi, 2006]. Additionally, lectins can act as antitumor agents inducing programmed cell death targeting apoptotic and autophagic pathways [Fu et al., 2011].

Dehulling and soaking have no effect on lectin levels [Embaby, 2010], however, and in contrast to the trypsin inhib-

itors, lectins are more heat-stable, and culinary techniques do not completely eliminate these toxic compounds unless they are cooked under high pressure [Pusztai et al., 1998; Embaby, 2010]. Therefore, limitation of HG intake is recommended due to the difficulties in their elimination and their harmful effects on health.

## NON-NUTRITIONAL COMPOUNDS OF NON-PROTEIN ORIGIN

### Phytic acid

Pulses are a good source of phytic acid (PA, myo-inositol-(1,2,3,4,5,6) hexakis-phosphate, IP<sub>6</sub>) that occurs in many seed plants usually in the form of soluble sodium or potassium salts [Kumar et al., 2010]. It is well-documented that PA blocks absorption of micronutrients such as P, Ca, Mg, Fe and Zn, and negatively affects the absorption of lipids and proteins. PA also inhibits important digestive enzymes such as amylase, pepsin and trypsin. For this reason, WHO has labeled PA as the main cause of anemia, an iron-deficiency disease. The reduced PA intake is of outstanding concern, mainly for people who consume large portions of grain legumes in their diets. PA is not digested by human and monogastric animals, due to the absence of phytase in the gas-

trointestinal tract. Therefore, cations linked with PA are not available and may disturb metabolic processes that require the presence of specified microelements, which can cause weakening of growth and rickets. On the basis of different studies on cation bioavailability four conclusions were formulated: *i.* bioavailability of cations is diversified and depends on the product; *ii.* the quality of legume seeds as the source of cations is limited by the biological availability of Zn; *iii.* legume native Zn (but not Fe) is biologically less available than the added inorganic Zn in a diet containing legumes, and *iv.* the availability of Zn in the ready-to-eat product depends on the molar ratio of PA [Lonnerdal *et al.*, 1989].

PA can also exert beneficial effects on human health. Scientific studies have reported a relationship between consumption of myo-inositol phosphates such as IP<sub>6</sub> from soybean and lower risk of cardiovascular disease and cancer [Campos-Vega *et al.*, 2010]. The PA mechanism of action is still unknown, however, anticarcinogenic activity of IP<sub>6</sub> has been linked to its ability to chelate metal ions [Steer & Gibson, 2002].

The studies of Chitra *et al.* [1995] on PA carried out in several genotypes of soybean, urd bean, mung bean, pigeon pea and chickpea showed that soybean is the richest source of PA (36.4 mg/g), followed by urd bean (13.7 mg/g), pigeon pea (12.7 mg/g), mung bean (12.0 mg/g) and chickpea (9.6 mg/g). On average, PA constitutes 78.2% of the total phosphorus content and this value was the highest for soybean (84.9%) and the lowest for mung bean (71.8%), data presented in Table 1. These results coincide with the studies carried out by other authors [Bastianelli *et al.*, 1998; Duc *et al.*, 1999; Singh, 1999; Wang *et al.*, 2009; Saghai Maroof *et al.*, 2009]. PA is stored in dormant seeds as a source of phosphorous (60.90% of the total phosphorous) and its content in legumes depends on the botanical species and variety, growing conditions and phosphate fertilizers, as most important factors [Thavarajah *et al.*, 2010].

Different procedures are being used for the partial or total reduction of PA: decortications, soaking, cooking, germination, fermentation, enzymatic degradation by means of phytase, utilization of the variable solubility of protein-phytinian complex at an acidic pH, ultrafiltration at controlled pH and thermal treatments [Elmaki *et al.*, 2007; Sangronis & Machado, 2007; Liang *et al.*, 2008; Khattab & Arntfield, 2009; Wang *et al.*, 2009; Kumar *et al.*, 2010]. Phytases synthesized by *Aspergillus oryzae*, *Rhizopus chinensis* and *R. oligosporus*, *Neurospora*, *Mucor dispersus*, that are applied for the production of oriental dishes, are implicated in the enzymatic degradation of PA. The use of commercial phytase leads to a noticeable diminution in PA of peas and lentils, whereas lower inositol phosphates with less chelating properties are released [Frias *et al.*, 2003b]. Methods using water extraction and diversified solubility involve the preparation of water-soluble extracts of legume proteins and slow adjustment to a pH, at which one component of the protein-phytinian complex is insoluble. The ultrafiltration method at a controlled pH also achieved effective results [Hurrell *et al.*, 2003; Kumar *et al.*, 2010; Ali *et al.*, 2011]. Traditional processing, such as dehulling, soaking and cooking, significantly increases the level of PA in bitter and sweet lupine seeds [Embaby, 2010]. On

the other hand, 6 days of germination, soaking and cooking lead to a decrease of PA content in lentil seeds [Vidal-Valverde *et al.*, 1994]. Rasha *et al.* [2011] demonstrated that kidney beans showed the greatest reduction in PA of 85.4%, compared to 77.0% for soybeans and 69.3% for mung beans after fermentation with *Lactobacillus bulgaricus* for 72 h.

### Raffinose Family Oligosaccharides

The raffinose family oligosaccharides (RFOs), also called  $\alpha$ -galactosides, belong to low-molecular-weight non-reducing saccharides that are widespread in the plant kingdom. *Fabaceae* family plants are rich sources of RFOs [Gulewicz, 1998; Muzquiz *et al.*, 1999]. The RFOs are found in seeds, leaves, roots and tubers in different plant families. In seeds, they perform very important physiological functions in plant acclimation related with acquisition of desiccation tolerance during seed development and maturation and seed longevity during dry storage [Obendorf & Gorecki, 2012].

RFOs are  $\alpha$ -(1-6)-galactosides linked to the C-6 carbon of the glucose moiety of sucrose [Kadlec, 2001]. A representative of this group is the trimer, raffinose. The higher homologues of raffinose are stachyose (tetramer), verbascose (pentamer), ajugose (hexamer) and unnamed longer-chain oligosaccharides up to nonamer. RFOs occupy a second place after saccharose in abundance as water-soluble carbohydrates and, therefore, they can be water-leached.

RFOs biosynthesis in legumes started to appear in the seed development at 37, 40 and 45 days after flowering, respectively, in the *Pisum sativum* L. cv. Ergo, *Vicia faba* ssp. minor Harz., cv. Tibo and *Lupinus luteus* L. cv. Juno [Frias *et al.*, 1996a]. The accumulation of RFOs increases with the development of seeds and the highest concentration in the mature seeds was found at 3.8% in peas, 4.5% in faba beans and 10.4% in lupines.

The RFOs are not hydrolyzed by digestive enzymes in the human gastrointestinal tract and, therefore, they reach the small intestine and colon of human and monogastric animals intact. For this reason, they are also included in the non-digestible oligosaccharides or dietary fiber. In the colon, the oligosaccharides are fermented only by indigenous residents, some beneficial (*Bifidobacterium* and *Lactobacillus*) and other pathogenic and harmful microflora. Gases like hydrogen, carbon monoxide and carbon dioxide, methane and also organic acids are the products of oligosaccharide fermentation by pathogenic and harmful bacteria, contributing to the flatulence problem associated with legume consumption.

In contrast, the beneficial bacteria metabolize RFOs to organic acids, mainly lactic and acetic acids [Tomamatsu, 1994]. The difference in the metabolism of oligosaccharides by beneficial, pathogenic and harmful bacteria is presented in Figure 1.

In the past, RFOs were considered as the main flatus-causing compounds. However, they exert beneficial effects stimulating the growth and activity of bifidobacteria and lactobacilli in the human gut [Gulewicz, 1998; Kaplan & Hutkins, 2000; Rada *et al.*, 2002; Gulewicz *et al.*, 2002; Gulewicz & Pilarski, 2005; Sendra *et al.*, 2008; Martinez-Villaluenga *et al.*, 2008a; Zduńczyk *et al.*, 2010; Bednarczyk *et al.*, 2011; Muzquiz *et al.*,



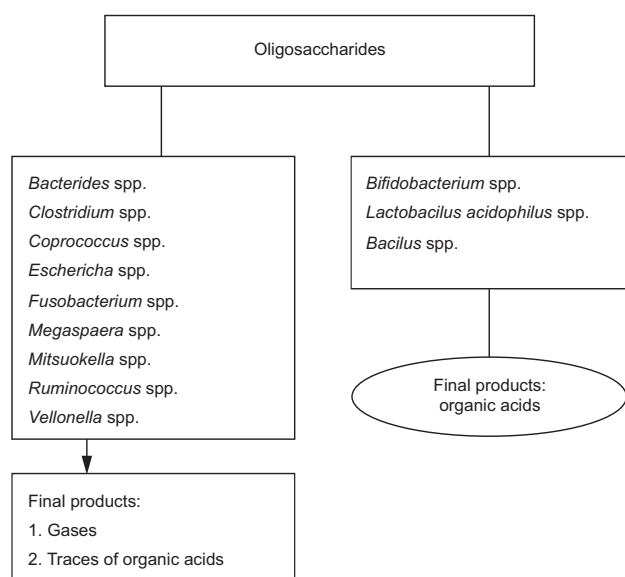


FIGURE 1. The metabolism of oligosaccharides in colon [Tomomatsu, 1994].

2012]. The oligosaccharides are a source of carbon and energy, which activate genes involved in sucrose utilization by bifidobacteria, inducing multiplication and processes of sugar fermentation [van Loo *et al.*, 1999; Trindade *et al.*, 2003]. The stimulating effect of these compounds on bifidobacteria growth and/or activity is known as the bifidofactor, which is accompanied with changes in quantitative and qualitative microbial composition leading to a number of beneficial metabolic changes in the human gut [Trafalska & Grzybowski, 2006]. In addition scientific evidence supports a relationship between the prebiotic activity of RFOs and other beneficial physiological effects including enhanced mineral absorption [Kobayashi *et al.*, 2006], stimulation of the immune system [Zheng *et al.*, 2012], regulation of lipid metabolism [Chen *et al.*, 2010], and attenuation of oxidative stress [Wang *et al.*, 2008b].

The RFOs content in legume seeds has been the object of many studies. It has been found that the content of RFOs varies not only among species, but also among varieties and cultivars of legumes. This statement was confirmed by results obtained by different authors [Bastianelli *et al.*, 1998; Duc *et al.*, 1999; Muzquiz *et al.*, 1999; Kadlec *et al.*, 2001; Kozłowska *et al.*, 2001; Martinez -Villaluenga *et al.*, 2005; Han & Baik, 2006].

Since these sugars are heat-stable compounds, different methods have been applied for RFOs reduction: extraction with n-butanol:HCl (92:8) at pH 4.5, extraction with 48% ethanol [Gulewicz *et al.*, 2000], germination, ultrasonic soaking (47 MHz), soaking with high hydrostatic pressure (621 MPa) and subsequent cooking [Aranda *et al.*, 2001; Han & Baik, 2006], and fermentation that is used more frequently in exotic non-conventional grain legumes, in which the RFOs are hydrolyzed by bacterial  $\alpha$ -galactosidase. Han & Baik, [2006] showed that soaking reduced largely RFOs in several legumes, such as soybean, peas, lentils and chickpeas. Soaking with ultrasound for 3 h and with high hydrostatic pressure for 1 h were the most effective for RFOs removal. In the case

of lentils, the cooking of presoaked seeds evidently led to a reduction of RFO content. This effect was not observed for other legumes. However, germination seems to remove most effectively RFOs in legumes [Peterbauer *et al.*, 2001; Martín-Cabrejas *et al.*, 2008]. Additionally, the addition of exogenous  $\alpha$ -galactosidase into legume flour blends has also been proved an effective method to reduce RFOs. The addition of commercial enzyme to lentil and peas has been shown to cause a decrease of 61–68 and 41–48% of raffinose, 80–85 and 67–91% stachyose and 100 and 95% verbascose, respectively [Frias *et al.*, 2003a].

## Tannins

Tannins are polyphenols which are relatively abundant in most of legumes and found mainly in coloured hulls of seeds. Their physiological functions consist in plant protection against the detrimental action of insects and microorganisms [Wilska-Jeszka, 2007]. They have a molecular weight ranging from 500 to 3000 Da, and possess the ability to form strong complexes with proteins and other polymers in the proper conditions of concentration and pH [Hassanpour *et al.*, 2011].

Tannins are compounds with diversified chemical structure as well as physical and biological properties. In legumes, they mainly belong to two groups of polyphenolic compounds: *i.* tannic acid type known as hydrolyzable tannins (HT) and *ii.* catechin type also referred as condensed tannins (CT) and proanthocyanidins (PRA). Their hydroxyl groups are partially or totally esterified and bound with the residue of gallic acid or its derivatives. HT are easily hydrolyzed by weak acids, bases or enzymes to form monomeric products. Degradation products of HT are toxic and can cause poisoning to organisms. In contrast, non-hydrolyzable tannins (CT and PRA) are derivatives of flavonoids – a diversified group of secondary plant metabolites with the characteristic carbon skeleton C6-C3-C6 without the sugar units [Guimarães-Beelen *et al.*, 2006].

Naturally occurring legume tannins are reported to interact with both enzymes and non-enzymatic proteins to form tannin-protein complexes, which results in the inactivation of digestive enzymes and, consequently, protein insolubility. *In-vitro* and *in-vivo* studies have shown that legume tannins decrease protein digestibility either by inactivating digestive enzymes or by reducing the susceptibility of the substrate proteins after forming complexes with tannins and absorbing ionizable iron. The presence of tannins in foods also affects the availability of vitamins A and B<sub>12</sub> [Wang *et al.*, 2000]. On the other hand, many researchers demonstrated that tannins can have positive effects on animals exerting antimicrobial, anthelmintic and protein-bypassed effects in ruminants [Tandon *et al.*, 2008; Waghorn, 2008; Hassanpour & Baghbani Mehmandar, 2012]. In addition, tannins show multiple actions in foods. They form the taste and colour and show antioxidant activity that stabilizes fats and other labile food ingredients. Drużyńska & Klepacka [2004] showed that the preparations from white, black and pink bean hulls containing mainly condensed tannins possess antioxidant properties. Simultaneously, extracts from black and pink bean hulls showed higher radical-scavenging ability than

those from the white hulls. In addition, these extracts showed higher antioxidant activity to hydroxyl radicals in the reactions catalyzed by a transition element. Positive correlations have been reported between dark hull colour and antioxidant activity in legume seeds [Dueñas *et al.*, 2006; Buyukcapar & Kamalak, 2007; Oomah *et al.*, 2011].

The content of tannins in legume seeds depends on many factors, such as genetic and environmental conditions during plant growing and post-harvest storage. Tannin content ranges from 45 to 2000 mg/100g. The highest tannin content is found in faba bean, followed by black gram, mung bean, horse gram, kidney bean, moth bean, peas, pigeon peas and, finally, soybean [Bastianelli *et al.*, 1998; Duc *et al.*, 1999; Caldas & Blair, 2009; Avola *et al.*, 2009; Wang *et al.*, 2009].

Studies carried out by Goel *et al.* [2005] and Waghorn [2008] showed that deleterious effects of legume tannins on animals are related to lower feed efficiency and growth depression of experimental animals, results that can be extrapolated to human beings. For this reason, tannins occurring in legumes should be reduced by processing using one or combined methods, such as dehulling, soaking, cooking, germination, fermentation, enzyme addition or vaporization of seeds over a long time. It has been shown that soaking of beans in sodium bicarbonate or in mixed salt solutions was a more effective procedure for removing tannins from dry beans than soaking in water, conducting to a reduced cooking time. In contrast, Embaby [2010] reported that traditional household soaking and cooking did not affect tannin levels in both sweet and bitter lupine cultivars. Soaking followed by cooking treatments significantly decreased tannin content. In addition, conventional cooking and autoclaving in a pressure cooker were the most effective methods in both seeds. However, dehulling followed by soaking and cooking resulted in a significant increase of tannin content that was explained by tannin penetration into cotyledons and protein binding during processing.

Over the last few years a great attention has been focused on the degradation of tannins by means of tannase produced by microorganisms belonging to filamentous fungi, yeast and bacteria [Pleszczyńska & Szczodrak, 2005]. Utilization of this method on a large scale is limited so far by production costs. Additionally, the stability of enzymes and their utilization in the immobilized form require further investigation. Nevertheless, fermentation of *Vigna sinensis* with natural microbiota and *Lactobacillus plantarum* modified the content of phenolic compounds bringing about an increase in the antioxidant activity and further autoclaving of fermented beans enabled production of food with higher functionality [Dueñas *et al.*, 2005].

## Alkaloids

### Quinolizidine Alkaloids (QA)

Alkaloids are heterocyclic and basic organic compounds mainly of the plant origin. They occur in plants in the form of salts of organic acids such as oxalic acid, hydroxysuccinic acid, citric acid, succinic acid or tannic acids. These compounds show a strong physiological action on human and animals stimulated through narcotic to toxic. Quinolizidine alkaloids (QA) are the main harmful NNCs occurring in *Lupinus* species.

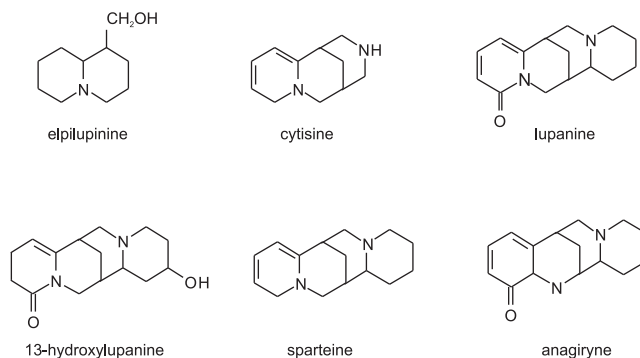


FIGURE 2. Structures of lupine alkaloids [Gulewicz, 1988].

Although the knowledge on the alkaloid composition of different lupine species has been enriched in the last few years due to cutting edge methods (HPLC, GC, GCMS), there is still a lack of information about their full composition.

Lupine alkaloids belong to two groups: piperidine alkaloids and quinolizidine alkaloids, the latest one including bis-quinolizidine and tricyclic quinolizidine alkaloids [Liebisch & Schutte, 1985]. The structures of lupine alkaloids are presented in Figure 2. The alkaloid composition is characteristic for each species and is often used as the taxonomic criterion. Therefore, different lupine varieties from the same species accumulate very similar or identical forms of alkaloids, although their concentrations can be different.

Alkaloids are chemical weapons and play very important physiological functions in lupines: allelopathic functions in the plant-plant effect, bacteriostatic function in the plant-bacteria effect and herbivore function in the plant-consumer effect [Wink, 1998].

The seed of modern cultivars of *Lupinus angustifolius* normally contain less than 0.03% alkaloids. The acute oral LD<sub>50</sub> of a pro rata mixture of the alkaloids of *L. angustifolius* seed was found to be 2279 mg/kg in rats. For lupanine, the LD<sub>50</sub> administrated orally and intraperitoneally was 1464 mg/kg and 177 mg/kg, respectively. For 13-hydroxylupanine, the LD<sub>50</sub> by intraperitoneal injection was 199 mg/kg. Lupanine and 13-hydroxylupanine constitute about 85% of the total alkaloids in lupin. It is suggested that they do not pose a health problem for humans since their consumption is low and they can be quickly eliminated [Pettersson *et al.*, 1987]. Nevertheless, trembling, shaking, excitation and convulsions characterize the poisoning of mammals by QA intoxication. The health authorities of some countries have decided to regulate the QA content in lupin flours and foods by fixing the maximum limit to 200 mg/kg [ACNFP 1996; Bulletin 1998, ANZFA, 2001].

Seeds of various species of lupines have been used as food for over 3000 years around the Mediterranean area and for as much as 6000 years in the Andean highlands [Uauy *et al.*, 1995]. Consumers are used to soak the seeds in water to remove most of the bitter and toxic alkaloids and then cook or roast the seeds to make them edible. An efficient method for the removal of alkaloids has been described by Gulewicz [1991a,b]. It consists in soaking lupine seeds in water

and subsequent ethanol or methanol extraction of previously crushed lupine seeds or lupine seeds soaked in water. The final products of these methods are: protein concentrate, high dietary fiber and lupine extract showing yield-increasing activity [Ciesiolka *et al.*, 2005]. As revealed in the studies of Gulewicz *et al.* [2002], the lupine extract is also a rich source of RFOs with prebiotic properties.

An alternative method involving the addition of coagulating proteins to an aqueous medium for debittering lupine (*Lupinus luteus* L.) has also been used [Chango *et al.*, 1993] where the proteins solubilized in an aqueous medium containing alginic acid were coagulated in a bath of calcium chloride. Jimenez-Martinez *et al.* [2010] also used aqueous acid and alkaline thermal treatments for *Lupinus campestris* debittering. The alkaline treatment appeared as the most effective method for the elimination of QAs. This method allowed also the reduction of other NNCs, which enabled achieving protein-rich products. In respect of sensorial properties, aqueous soaking at room temperature was a more favorable process than alkaline and thermal debittering methods in the production of lupine bean snack from *Lupinus albus* L. seeds [Erbas, 2010].

#### Pyrimidine glycosides – vicine and convicine

Cotyledons of horse bean (*Vicia faba*) seeds contain two alkaloids of the pyrimidine glycoside group: vicine and convicine. These compounds cause hemolytic anemia called favism, which is harmful to the intolerant consumers. It is usually an ethnic disease and concerns people whose erythrocytes are deficient in glucose-6-phosphate dehydrogenase (G6PD). The seeds of horse bean provide around 0.4–1.0% of vicine and about 0.3% of convicine. These glycosides occur in cotyledons of seeds of species *Vicia faba* and constitute about 2% (for vicine) and about 0.3% of dry weight (for convicine). The discovery of the mutant *allele vc*, which lead to 10 to 20-fold reduction in vicine and convicine contents, has been a significant recent achievement [Gutierrez *et al.*, 2006] and, hence, nowadays research studies are conducted to evaluate the safety of low vicine and convicine cultivars in humans [Crepon *et al.*, 2010].

#### Saponins

Saponins are a diverse group of low molecular-weight NNCs that are widely distributed throughout the plant kingdom. They belong to surface-active sterol glycosides or triterpene glycosides. These compounds possess hemolytic properties, however, oral administration of saponins is practically harmless for humans. The acute toxicity of different saponins LD<sub>50</sub> ranges from 50 to 3000 mg/kg for mice [Oakenfull, 1981]. Unfortunately, studies on toxicity did not include the saponins of edible legumes and little is known about the structures of saponins occurring in legumes. As an example, in the soybean seeds, there are five non-polar sapogenins which can bind monosaccharides like galactose, arabinose, rhamnose, glucose, xylose and glucuronic acid [Krishnamurthy *et al.*, 2012]. The content of saponins in legume seeds is collected in Table 1. Defatted soy flour has the highest saponin content (0.58%) followed by navy beans (0.32%) and kidney beans (0.29%). In six species of lupine saponin content ranged from null in the *L. albus* to 470 mg/kg in *L. angustifolius* [Bastianelli

*et al.*, 1998; Kadlec *et al.*, 2001; Kozłowska *et al.*, 2001; Gurfinkel & Rao, 2002].

The legume saponins have health promoting effects in the gastrointestinal tract since they are involved in the reduction of cell fission rate of mucous membrane and, consequently, prevent cancer in the large intestine. The daily dose of saponins recommended by dieticians is 200 mg [Shi *et al.*, 2004]. In addition, the presence of saponins in the diet seems to decrease the risk of heart disease as they have been shown to express blood cholesterol-lowering properties [Shi *et al.*, 2004] and protective effect on myocardium [Chu *et al.*, 2013].

Saponins belong to the soluble, heat-sensitive compounds and during soaking and cooking, portions of saponins are dissolved in water and lost in the soaking, washing, and cooking liquids. Thermal processes can increase the stability of saponins and ensure their preservation in canned bean products, therefore, it is worth improving thermal processing technology to increase the quality of bean products [Shi *et al.*, 2004].

### EFFECT OF PROCESSING ON THE NUTRITIVE QUALITY OF LEGUMES

The choice of the treatment method for the elimination/reduction of NNCs in legumes without deterioration of their nutritive value has been a thick research topic for many researches during the last thirty years. The effectiveness of the processing method used depends not only on the type and variety of legume, but also on the chemical properties of the NNCs and conditions and intensity of the selected processing technique.

#### Germination

Germination is one of the most effective methods to improve the nutritional quality of legumes and to reduce the adverse NNCs. Germination mobilizes reserve nutrients required for plant growth hydrolyzing proteins and storage carbohydrates to find the required substrates for the seed development. During germination, seed enzymatic systems activate and protease activity increases [Urbano *et al.*, 2005] and, consequently, protein nitrogen decreases, whilst peptides, polypeptides and non-protein amino acids increase [Kuo *et al.*, 2004; Martinez-Villaluenga *et al.*, 2006b; Rodriguez *et al.*, 2007], thereby improving protein quality [Urbano *et al.*, 2005; Ghavidel & Prakash, 2007]. Goyoaga *et al.* [2011] described the greatest protein mobilization of *Vicia faba* legumes by hydrolysis after 3 days of germination. Hydrolysis and utilization of carbohydrates also occur during germination and starch digestibility rises in conventional and non-conventional legumes [Frias *et al.*, 1998; Benitez *et al.*, 2013; Martin-Cabrejas *et al.*, 2008]. At the same time, contents of some other nutrients like minerals and vitamins, such as B-group vitamins and ascorbic acid as well as simple sugars, are increased. Furthermore, germination also improves the antinutritional capacity [Frias *et al.*, 2005] and imparts better organoleptic properties to seed sprouts [Torres *et al.*, 2007].

The effect of germination of three varieties of pea seeds (*Pisum sativum*) on protein Osborne fractions and amino acid content was investigated by Martinez-Villaluenga *et al.* [2008b]. The germination process led to an increase of to-



tal protein in all tested varieties although it was diversified. Significant changes were observed in the protein quality and quantity composition of particular fractions. It concerned the albumin fraction that is composed, among others, of undesirable proteins like: trypsin inhibitor, lectins, lipoxygenase, antigenic and allergenic compounds that decrease the nutritional quality and safety. Germination caused a significant decrease of this fraction. All protein fractions were characterized by a higher essential amino acid content in comparison to raw seeds. Germination also improved essential amino acid indexes (EAAI adult and EAAI egg). The above results were confirmed by studies on the influence of germination on protein fractions and their amino acid composition in different lupine species and varieties [Gulewicz *et al.*, 2008; Chilomer *et al.*, 2010]. Moreover, it was shown that germination caused a decrease of alkaloids and RFOs content [Chilomer *et al.*, 2010].

PA decreases during lentil germination since naturally occurring phytases are activated and phytate is progressively degraded [Egli *et al.*, 2002], whilst lower phosphorylated inositol phosphates are formed [Doblado *et al.*, 2005], leading to a decrease in its mineral-binding strength [Sandstrom & Sandberg, 1992] and, hence, to mineral bioavailability [Urbano *et al.*, 2005; Ghavidel & Prakash, 2007]. Vidal-Valverde *et al.* [2002] showed phytate degradation to lower inositol phosphate forms (penta- to triphosphates) using germination periods of 2, 4 and 6 days with or without light. In order to maximize the beneficial effects of germination on the content of nutrients and non-nutritive compounds, these authors conclude that the germination process should be carried out in darkness for 6 days. Germination also seems to reduce hemagglutinating activity possibly due to proteolysis of lectins during germination [Kovalchuk, 2006]. The reduction of RFOs during seed germination has been widely reported leading to better legume digestibility and lower expected flatus production [Frias *et al.*, 1996b; Oboh *et al.*, 2000; Martin-Cabrejas *et al.*, 2008]. Protease inhibitors (PI) suffer a noticeable reduction of their activity as a consequence of germination. Frias *et al.* [1995] found a progressive but slow decrease (12%) on the PI activity after 6 days of lentil germination, followed by a faster decrease (45%) after 10 days. In the same line, a sharp reduction  $\alpha$ -amylase inhibitor activity was reported related to a higher potential *in vitro* antidiabetic effect and expected glycemic index in lentil sprouts after 3 days of germination [Świeca *et al.*, 2013]. Lopez-Amoros *et al.* [2006] found that germination modifies the quantitative and qualitative phenolic composition of pea, lentils and beans, and the changes depend on the type of legume and the germination conditions. The effect of germination and seedling growth on the content and distribution of vicine and convicine of two *Vicia faba* L. varieties was determined by Burbano *et al.* [2008] and the results showed that vicine and convicine slowly declined in cotyledons, while in the embryo axis, vicine levels were sharply reduced and the convicine amount was slightly increased.

Urbano *et al.* [1995] showed that germination led to the total elimination of RFOs and a considerable increase of thiamin, riboflavin, and niacin content of lentils, a reduction of the total starch content and the PI activity. A comparison between the processing methods carried out by Vidal-

-Valverde *et al.* [1998] showed that germination appeared to be the best choice to reduce RFOs and PA and to provide appreciable increments of dietary fiber, Ca and starch.

Vitamin content seems to be also changed during germination process and while thiamine seems to decrease slightly after 6 days, riboflavin and niacin increased sharply as a consequence of germination [Urbano *et al.*, 1995; Prodanov *et al.*, 1997; Vidal-Valverde *et al.*, 2002]. Similarly, it has been reported that legume sprouts provide a larger quantity of vitamin C and E than ungerminated seeds [Frias *et al.*, 2005; Torres *et al.*, 2007; Doblado *et al.*, 2007; Fernandez-Orozco *et al.*, 2008a]. Consequently, the antioxidant capacity is enhanced [Frias *et al.*, 2005; Fernandez-Orozco *et al.*, 2008b]. These results confirm that germination enhances the nutritional value of legumes and satisfies the consumer's demands for fresh, nutritious and healthy foods.

Germination can also be utilized as seed pre-treatment before some concomitant processes, such as cooking or dry heating to preserve these perishable foods. In this sense, high hydrostatic pressure has elongated the shelf-life of mung bean sprouts [Doblado *et al.*, 2007; Peñas *et al.*, 2010] and improved soybean sprout antigenicity [Peñas *et al.*, 2011].

### Heat treatment

Adamidou *et al.* [2011] evaluated the influence of extrusion at various pre-treatments and dryer temperatures of field peas, chickpeas, and faba beans on both nutritional quality and adverse NNCs. They showed that extrusion of chickpea and field pea did not have a drastic effect on RFOs values. The pre-treatment considerably reduced PI levels and, consequently, improved the nutritional value. Similarly, phytate and total tannin contents were greatly reduced irrespective of the pre-treatment and drying, suggesting that the combination of wet pre-treatment with extrusion can improve the nutritional value of legumes. On the other hand, only drying at 90–150°C without any pre-treatment does not significantly further NNCs levels.

Studies carried out by Frias *et al.* [2011] on the effect of extrusion at 129°C, 135°C and 142°C on the nutritional quality of pea (*Pisum sativum* L) showed that this thermal process slightly increased protein and fat contents and led to reduced levels of dietary fiber, thiamine, RFOs and negligible PI activity. The extrusion had no impact on the NPU, NPR, CRNPR, TPD, and BV indexes used to determine the pea protein quality. On the other hand, the authors showed that the temperature of 135°C was the most advantageous and recommended for the manufacture of food products with a high nutritive value.

Carbonaro *et al.* [2000] studied the effect of cooking common bean (*Phaseolus vulgaris* L) and faba bean (*Vicia faba* L) on the *in-vitro* digestibility (IVPD) of raw protein and their protein fractions in growing rats as a model of humans. Thermal treatment only caused slight reductions of PI and HG. The IVPD of raw common bean flour was 72.4% and the cooking had no effect on its level. In contrast, the IVPD of faba bean decreased from 86.5 to 60.6% as a result of cooking. *In-vitro* digestibility of globulins extracted from both species was at 70%. Processing (mild hydrothermal treatment and the addition of phytase) of pea flour was evaluated in growing rats in respect of its nutritive quality by Ur-



bano *et al.* [2003]. The mild hydrothermal treatment caused a significant increase in the digestive utilization of protein and in the metabolic utilization of protein and carbohydrates. On the other hand addition of phytase did not have an effect on these parameters.

Investigations on the effects of thermal treatment, roasting and boiling, on the content of vicine and convicine in mature cotyledons of 10 varieties of Mexican *Vicia faba* L. showed that raw cotyledons contained 2.88–6.10 and 0.63–1.68% vicine and convicine, respectively. After roasting and boiling, a 12% and 40% and 30% and 61% decrease was observed in vicine and convicine contents, respectively [Cardador-Martínez *et al.*, 2012].

### Fermentation

Fermentation is one of the oldest and most economical methods of food processing and preservation. Fermentation can be spontaneously initiated with the microbiota naturally present in the legume (spontaneous fermentation) or controlled by the use of specific starters. Legumes are fermented to improve their sensory characteristics, such as flavor and taste, and to increase the amount and availability of nutrients [Svanberg & Lorri, 1997; Granito *et al.*, 2002]. This can be achieved by degradation of non-desirable NNCs by the hydrolysis of certain food components and by the synthesis of some nutritious promoters [Frias *et al.*, 1996a]. In addition, by increasing the titratable acidity and reducing the pH of the fermented food to levels below 4.5, fermentation precludes the proliferation of contaminating acid-intolerant species of bacteria and fungi, preserving the fermented legumes for longer periods [Fernandez-Orozco *et al.*, 2007].

Changes occurring during the fermentation process are mainly due to endogenous enzymes of the seed and the enzymatic activity of the microbiota present in the legumes. Differences in the final nutritional value of the fermented product depend on the type of the induced microorganism as well as whether the whole seed or flour suspensions with different ratios of water were used [Kozłowska *et al.*, 1996; Fernandez-Orozco *et al.*, 2008b]. Fermentation can be combined with other culinary (soaking and cooking) and technological (germination prior to the fermentation process) treatments to further remove these adverse compounds and improve the nutritional value and functionality of the fermented product [Torres *et al.*, 2006].

Natural and spontaneous fermentation of lentils for 4 days at 42°C led to a sharp reduction in the trypsin inhibitor activity and condensed tannins; however, there were slight increases in the total nitrogen content and *in-vitro* protein digestibility of the fermented products [Tabera *et al.*, 1995]. In similar fermentation conditions, Cuadrado *et al.* [2002] found a considerable decrease in the levels of lectins and phytic acid and, hence, which resulted in the increase of the nutritional potential of the final product. Similarly, Dueñas *et al.* [2005] observed beneficial changes in phenolic compounds of *Vigna sinensis*, which were correlated with enhanced antioxidant activity.

After natural fermentation of whole kidney seeds for 96 h at 35°C, Granito *et al.* [2002] found a reduction in pH, in the content of trypsin inhibitors, phytic acid and RFOs,

as well as a considerable increase of riboflavin and available starch. The removal of RFOs by natural fermentation has been corroborated in other legumes, such as *Vigna sinensis* [Doblado *et al.*, 2005] or *Cajanus cajan* [Torres *et al.*, 2006], under condition of providing glucose to induce fermentation. Natural and induced fermentation led to a sharp reduction of phytic acid, and to an increase in lower inositol phosphates [Kozłowska *et al.*, 1996; Doblado *et al.*, 2003]. Induced solid and liquid state fermentation of soy beans for 48 h at 30°C led to a sharp reduction in the IgE immunoreactivity and to a significant increase in most of the essential amino acids [Frias *et al.*, 2008]. Inclusion of fermented soybean meal in a pig diet resulted in great performance and less diarrhea than in the animals fed with soybean meal, as well as in reduced immunoreactivity due to partial hydrolysis of allergenic proteins during fermentation [Song *et al.*, 2010].

### Dehulling and its combined treatments

Dehulling is one of the easiest methods used for processing legumes in developing countries and is often combined with some other methods, such as soaking, cooking and roasting. Belal *et al.* [2011] evaluated the effects of dehulling, roasting, and enzyme treatment on the nutritive quality of cowpea seeds and found that dehulled and dehulled roasted seeds significantly increased the feed intake, body weight gain and protein intake in broilers. On the other hand, Abdelatif & El-Jasser [2010] showed that traditional processing (soaking and cooking) decreased the PI activity and improved the protein digestibility of cowpea seeds, but the biological value (BV) and net protein utilization (NPU) were not affected.

In addition, Embaby [2010] observed *in vitro* protein digestibility improvements for both sweet and bitter lupine after conventional processing methods such as soaking, dehulling, cooking, in comparison to raw material. Wang *et al.* [2008a] when using culinary methods such as soaking, cooking and dehulling on different pea seeds stated that the plant genetics plays a significant effect on contents of crude protein, starch, ash, total dietary fiber, soluble and insoluble dietary fiber, minerals, sucrose and the NNCs PI, PA and RFOs. In soaked and cooked pea seeds, an increase was observed in protein content, total and insoluble dietary fiber, Ca, Cu, Mn and P, whilst on the other hand, a reduction was noticed for contents of ash, Fe, K, Mg, Zn, sucrose and oligosaccharides [Embaby, 2010].

Dehulling led to the increase of crude protein, starch, K, P, PA, and RFOs content, but to a reduction of total, soluble and insoluble dietary fiber and minerals such as Ca, Cu, Fe, Mg and Mn. In weaning pigs, Emiola & Gous [2011] showed that diets may contain as much as 300 g of dehulled faba bean/kg without any harmful influence on growth rate, feed intake, feed conversion efficiency or time needed to achieve the final expected weight.

### Enzyme and extraction treatments

The nutritive quality of processed lupin (*Lupinus angustifolius*) meal treated with  $\alpha$ -galactosidase and extraction with ethanol for the removal of RFOs was evaluated in rainbow trout feeding [Glencross *et al.*, 2003]. The results of the studies showed that processing based on ethanol extraction had

the greatest effect on its apparent digestibility, especially the nitrogen, organic matter and nitrogen-free extractive components. A significant difference in the nitrogen digestibility and improvements of most nutrient parameters was observed when exogenous  $\alpha$ -galactosidase was added to the lupin meal. Similarly, the removal of RFOs by treatment of legume flours with exogenous  $\alpha$ -galactosidase had beneficial nutritional implications [Doblado *et al.*, 2003] and the addition of commercial phytase led to a sharp decrease of phytic acid [Frias *et al.*, 2003b].

Solvent extraction also affected the removal of some NNCs. Studies conducted by Martinez-Villaluenga *et al.* [2006a] on the effects of white and yellow lupine seeds after processing (alcohol extraction) on different nutritional and non-nutritive compounds concluded that processed lupine seeds with low RFOs content were important nutritional products containing high contents of protein, dietary fiber and fat. Moreover, processed seeds contained acceptable levels of thiamin, riboflavin and vitamin E. On the other hand, Jimenez-Martinez *et al.* [2010] demonstrated that debittering of *Lupinus campestris* seeds by aqueous, acid, and alkaline treatments resulted in an increase of total proteins by 26, 31, and 32%, respectively, in comparison with untreated seeds. The best protein quality was obtained in samples after alkaline treatment whereas the apparent digestibility was over 90%. Extraction of lentil flours with 80% ethanol at different temperatures (25 and 50°C) resulted in an increase of the total nitrogen content. The content of protein or non-protein compounds and vitamin B<sub>1</sub> and B<sub>2</sub> depended on the extraction temperature. The processing did not have significant effects on amino acid composition and protein digestibility. However, there was a positive correlation between protein digestibility and lysine availability [Sanz *et al.*, 2001].

## CONCLUSIONS

Legume seeds are produced and consumed worldwide. They are sources of the energy, protein and other important nutrients consumed in developed and underdeveloped countries where legumes constitute a significant part of the diet. However, the presence of NNCs, which in some cases can be considered as bioactive compounds, limits the effectiveness of the high nutritional value of legumes. Therefore, both aspects should be balanced bearing in mind that their removal is essential for improving nutritional quality. Therefore, the modification of the concentration of these disadvantageous compounds can be achieved by conventional processing that is usually carried at the household milieu in order to improve the nutritional status of a large number of world citizens.

## REFERENCES

1. Abdelatif S., El-Jasser H., Chemical and biological properties of local cowpea seed protein grown in Giza region. *Int. J. Agric. Biol. Sci.*, 2010, 1 (2), 88–94.
2. ACNFP 1996. Report on seeds from narrow leafed lupin. Appendix IX MAFF Publications, London GB, pp.107.
3. Adamidou S., Nengas I., Grigorakis K., Nikolopoulou D., Jauncy K., Chemical composition and antinutritional factors of field peas (*Pisum sativum*), chickpeas (*Cicer arietinum*), and faba beans (*Vicia faba*) as affected by extrusion preconditioning and drying temperatures. *Cereal Chem.*, 2011, 88, 80–86.
4. Ali F., Mondor M., Ippersiel D., Lamarche F., Production of low-phytate soy isolate by membrane technologies: Impact of salt addition to the extract on the purification process. *Innov. Food Sci. Emer. Technol.*, 2011, 12 (2), 171–177.
5. Alonso R., Orue E., Marzo F., Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. *Food Chem.*, 1998, 63 (4), 505–512.
6. ANZFA (Australia New Zeland Food Authority) 2001. Lupin alkalids in food. A toxicological review and risk assessment. *Techn. Rep.*, Series 3, pp. 6–19 (<http://www.anzfa.gov.au>)
7. Aranda P., Dostalova J., Frias J., Lopez-Jurado M., Kozłowska H., Pokorný J., Urbano G., Vidal-Valverde C., Zduńczyk Z., Nutrition. 2001, *in: Carbohydrates in grain legume seeds. Improving nutritional quality and agronomic characteristics.* (ed. C.L. Hedley). CAB International, Wallingford, UK, pp. 61–87.
8. Avola G., Gresta F., Abbate V., Diversity examination based on physical, technological and chemical traits in a locally grown landrace of faba bean (*Vicia faba* L. var major). *Int. J. Food Sci. Tech.*, 2009, 44 (12), 2568–2576.
9. Barampama Z., Simard R.E., Nutrient composition, protein quality and antinutritional factors of some varieties of dry beans (*Phaseolus vulgaris*) grown in Burundi. *Food Chem.*, 1993, 47 (2), 159–167.
10. Bastianelli D., Grosjean F., Peyronnet C., Duparque M., Régnier J.M., Feeding value of pea (*Pisum sativum* L.) 1. Chemical composition of different categories of pea. *Anim. Sci.*, 1998, 67 (3), 609–619.
11. Bednarczyk M., Urbanowski M., Gulewicz P., Kasperczyk K., Maiorano G., Szwaczkowski T., Field and *in vitro* study on prebiotic effect of raffinose family oligosaccharides in chickens. *Bull. Vet. Inst. Pulawy*, 2011, 55, 465–469.
12. Belal N.G., Abdelati K. A., Albala S., Elawad S., Effect of dietary processed cowpea (*Vigna unguiculata*) seeds on broiler performance and internal organ weights. *Res. J. Anim. Vet. Sci.*, 2011, 6, 6–11.
13. Benítez V., Cantera S., Aguilera Y., Mollá E., Esteban R.M., Díaz M.F., Martín-Cabrejas M.A., Impact of germination on starch, dietary fiber and physicochemical properties in non-conventional legumes. *Food Res. Int.*, 2013, 50, 64–69.
14. Benjakul S., Visessanguan W., Thummaratwasak P., Isolation and characterization of trypsin inhibitors from some Thai legumes. *J. Food Biochem.*, 1999, 24, 107–127.
15. Birk Y., Plant Protease Inhibitors: Significance in nutrition, plant protection, cancer prevention and genetic engineering. Springer-Verlag Germany, 2003, pp 12–43.
16. Blair M.W., Sandoval T.A., Caldas G.V., Beebe S.E., Páes, M.I., Quantitative trait locus analysis of seed phosphorus and seed phytate content in a recombinant inbred line population of common bean. *Crop Sci.*, 2009, 49, 237–246.
17. Bulletin Officiel No98/27 du Conseil supérieur d'hygiène publique de France 1998
18. Burbano C., Cuadrado C., Varela A., Guillamón E., Pedrosa M.M., Goyoaga C., Muzquiz M., Content and distribution of vicine, convicine and l-DOPA during germination and seedling growth of two *Vicia faba* L. varieties. *Eur. Food Res. Technol.*, 2008, 227, 1537–1542.

19. Buyukcapar H.M., Kamalak A., Condensed tannin contents of some legume seeds used in fish nutrition. *J. Biol. Sci.*, 2007, 7, 74–76.
20. Caldas, G.V., Blair, M.W., Inheritance of seed condensed tannins and their relationship with seed coat color and pattern genes in common bean (*Phaseolus vulgaris* L.). *Theor. Appl. Genet.*, 2009, 119, 131–142.
21. Campos J.E., Whitaker J.R., Yip T.T., Hutchens T.W., Labra, A.B., Unusual structural character and complete amino acid sequence of a protease inhibitor from *Phaseolus acutifolius* seeds. *Plant Physiol. Biochem.*, 2004, 42, 209–214.
22. Campos-Vega R., Loarca-Piña G.F., Oomah B.D., Minor components of pulses and their potential impact on human health. *Food Res. Int.*, 2010, 43, SI, 461–482.
23. Carbonaro M., Grant G., Cappelloni M., Pustai A., Perspectives into factors limiting in vivo digestion of legume proteins: antinutritional compounds or storage proteins? *J. Agric. Food Chem.*, 2000, 48, 742–749.
24. Cardador-Martínez A., Maya-Ocaña K., Ortiz-Moreno A., Herrera-Cabrera B.E., Dávila-Ortiz G., Múzquiz M., Pedrosa M.M., Burbano C., Cuadrado C., Jiménez-Martínez C., Effect of roasting and boiling on the content of vicine, convicine and L-3,4-dihydroxyphenylalanine in *Vicia faba* L. *J. Food Qual.*, 2012, 35 (6), 419–428.
25. Chango A., Villaume C., Bau H. M., Nikolas J.P., Mejean L., Debitting of lupin (*Lupinus luteus* L) protein by calcium alginate and nutritional evaluation. *J. Sci. Food Agric.*, 1993, 63 (2), 195–200.
26. Chen Y.W., Huang S.C., Lin-Shiau S.Y., Lin J.K., Bowman-Birk inhibitor abates proteasome function and suppresses the proliferation of MCF7 breast cancer cells through accumulation of MAP kinase phosphatase-1. *Carcinogenesis*, 2005, 26, 1296–1306.
27. Chen H., Liu L.J., Zhu J.J., Xu B., Li R., Effect of soybean oligosaccharides on blood lipid, glucose levels and antioxidant enzymes in high fat rats. *Food Chem.*, 2010, 119, 1633–1636.
28. Chilomer K., Zaleska K., Ciesiolka D., Gulewicz P., Frankiewicz A., Gulewicz K., Changes in the alkaloid,  $\alpha$ -galactoside and protein fraction content during germination of different lupin species. *Acta Soc. Bot. Pol.*, 2010, 79 (1), 11–20.
29. Chitra U., Vimala V., Singh U., Geervani P., Variability in phytic acid content and protein digestibility of grain legumes. *Plant Foods Hum. Nutr.*, 1995, 47, 163–172.
30. Chokshi D., Toxicity studies of Blockal, a dietary supplement containing Phase 2 Starch Neutralizer (Phase 2), a standardized extract of the common white kidney bean (*Phaseolus vulgaris*). *Int. J. Toxicol.*, 2006, 25, 361–371.
31. Chu H.-B., Li D.-M., Shi B., Huang K.-X., Xin J.-L., Effects of soybean saponin on NOX4 and p22phox expressions in diabetic rat myocardium tissues and its protective effect on myocardium. *Journal of Jilin University Medicine Edition*, 2013, 39 (2), 251–254.
32. Ciesiolka D., Gulewicz P., Matinez-Villaluenga C., Pilarski R., Bednarczyk M., Gulewicz K., Products and biopreparations from alkaloid-rich lupin in animal nutrition and ecological agriculture. *Folia Biol.-Krakow*, 2005, 53, 60–66. Suppl.
33. Crepon K., Marget P., Peyronnet C., Carrouee B., Arese P., Duc G., Nutritional value of faba bean (*Vicia faba* L.) seeds for feed and food. *Field Crops Res.*, 2010, 115, SI, 329–339.
34. Cuadrado C., Hajos G., Burbano C., Pedrosa M.M., Ayet G., Muzquiz M., Pustai A., Gelencser E., Effect of natural fermentation on the lectin content of lentils measured by immunological methods. *Food Agri. Immunol.*, 2002, 14, 41–49.
35. Doblado R., Frias J., Muñoz R., Vidal-Valverde C., Fermentation of *Vigna sinensis* var. carilla flours by natural microflora and *Lactobacillus* species. *J. Food Prot.*, 2003, 66, 2313–2320.
36. Doblado R., Zielinski H., Piskula M., Kozłowska H., Muñoz R., Frias J., Vidal-Valverde C., Effect of processing on the antioxidant vitamins and antioxidant capacity of *Vigna sinensis* var. Carilla. *J. Agric. Food Chem.*, 2005, 53, 1215–1222.
37. Doblado R., Frias J., Vidal-Valverde C., Changes in vitamin C and antioxidant capacity of raw and germinated cowpea (*Vigna sinensis* var Carilla) induced by high-pressure treatment. *Food Chem.*, 2007, 101, 918–923.
38. Drużyńska B., Klepacka M., Antioxidant properties polyphenolic preparations from seed cover of black, pink and white bean (*Phaseolus*). *Zywn.-Nauk Technol. Jakość*, 2004, 4, 69–78 (in Polish; English abstract).
39. Duc G., Marget P., Esnault R., Le Guen J., Bastianelli D., Genetic variability for feeding value of faba bean seeds (*Vicia faba* L.). Comparative chemical composition of isogenics involving zero-tannin and zero-vicine genes. *J. Agric. Sci.*, 1999, 133, 185–196.
40. Dueñas M., Fernandez D., Hernández T., Estrella I., Muñoz R., Bioactive phenolic compounds of cowpea (*Vigna sinensis* L). Modifications by fermentation with natural microflora and with *Lactobacillus plantarum* ATCC 14917. *J. Sci. Food Agric.*, 2005, 85, 297–304.
41. Dueñas M., Hernández T., Estrella I., Assessment of the in vitro antioxidant capacity of the seed coat and the cotyledon of legumes in relation to their phenolic contents. *Food Chem.*, 2006, 98, 95–103.
42. Duranti M., Grain legume proteins and nutraceutical properties. *Fitoterapia*, 2006, 77, 67–82.
43. Egli I., Davidsson L., Juillerat M.A., Barclay D., Hurrell R.F., The influence of soaking and germination on the phytase activity and phytic acid content of grains and seeds potentially useful for complementary feeding. *J. Food Sci.*, 2002, 67, 3484–3488.
44. Elmaki H.B., Abdel-Rahman S.M., Idris W.H., Hassan A.B., Babiker E.E., El-Tinay A.H., Content of antinutritional factors and HCl-extractability of mineral from white bean (*Phaseolus vulgaris*). Cultivars: Influence of soaking and/or cooking. *Food Chem.*, 2007, 100, 362–368.
45. Embaby H.E.-S., Effect of soaking, dehulling, and cooking methods on certain antinutrients and in vitro protein digestibility of bitter and sweet lupin seeds. *Food Sci. Biotechnol.*, 2010, 19(4), 1055–1062.
46. Emiola I.A., Gous R.M., Nutritional evaluation of dehulled faba bean (*Vicia faba* cv. Fiord) in feeds for weaner pigs. *South African Soc. Animal Sci.*, 2011, 41 (2), 79–86.
47. Erbas M., The effects of different debittering methods on the production of lupin bean snack from bitter *Lupinus albus* L. seeds. *J. Food Qual.*, 2010, 33 (6), 742–757.
48. Fernandez-Orozco R., Frias J., Muñoz R., Zielinski H., Piskula M.K., Kozłowska H., Vidal-Valverde C., Fermentation as a bioprocess to obtain functional soybean flours. *J. Agric. Food Chem.*, 2007, 55, 8972–8979.
49. Fernandez-Orozco R., Frias J., Zielinski H., Piskula M.K., Kozłowska H., Vidal-Valverde C., Kinetic study of the antioxi-



- dant compounds and antioxidant capacity during germination of *Vigna radiata* cv. Emerald, *Glycine max* cv. Jutro and *Glycine max* cv. Merit. Food Chem., 2008a, 111, 622–630.
50. Fernandez-Orozco R., Frias J., Muñoz R., Zielinski H., Piskula M.K., Kozłowska H., Vidal-Valverde C., Effect of fermentation conditions on the antioxidant capacity of *Lupinus angustifolius* cv. Zapaton. Eur. Food Res. Technol., 2008b, 227, 979–988.
  51. Filippetti A., Azadegan G.H., De Pace C., Breeding strategies for seed protein content and trypsin inhibitors inferred from combining ability and heterosis in test-crosses of *Vicia faba*. Plant Breeding, 1999, 118 (5), 411–416.
  52. Frias J., Diaypollan C., Hedlez C.L., Vidal-Valverde C., Evolution of trypsin+inhibitor activity during germination of lentils. J. Agr. Food Chem., 1995, 43 (8), 2231–2234.
  53. Frias J., Diaz-Pollan C., Hedley C.L., Vidal-Valverde C., Evolution and kinetics of monosaccharide, disaccharide and  $\alpha$ -galactosides during germination of lentils. Z. Lebensm. Unters. Forsch., 1996a, 202, 35–39.
  54. Frias J., Vidal-Valverde C., Kozłowska H., Górecki R., Honke J., Hedley C.L., Evolution of soluble carbohydrates during the development of pea, faba bean and lupin seeds. Z. Lebensm. Unters. Forsch. 1996b, 203, 27–32.
  55. Frias J., Vidal-Valverde C., Kozłowska H., Tabera J., Honke J., Hedley C.L., Natural fermentation of lentils. Influence of time, concentration and temperature on the kinetics of monosaccharide, disaccharide and  $\alpha$ -galactosides. J. Agric. Food Chem., 1996c, 44, 579–584.
  56. Frias J., Fornal J., Ring S.G., Vidal-Valverde C., Effect of germination on physico-chemical properties of lentil starch and its components. LWT – Food Sci. Technol., 1998, 31, 228–236.
  57. Frias J., Doblado R., Vidal-Valverde C., Kinetics of soluble carbohydrates by action of endo/exo  $\alpha$ -galactosidase enzyme in lentils and peas. Eur. Food Res. Technol., 2003a, 216, 199–203.
  58. Frias J., Doblado R., Antezana J.R., Vidal-Valverde C., Inositol phosphate degradation by the action of phytase enzyme in legume seeds. Food Chem., 2003b, 81, 233–239.
  59. Frias J., Miranda M.L., Doblado R., Vidal-Valverde C., Effect of germination and fermentation in the antioxidant vitamin content and antioxidant capacity of *L. albus* L. var. Multolupa. Food Chem., 2005, 92, 211–220.
  60. Frias J., Song Y. S., Martinez-Villaluenga C., Gonzalez de Mejía E., Vidal-Valverde C., Immunoreactivity and amino acid content of fermented soybean products. J. Agric. Food Chem., 2008, 56, 99–105.
  61. Frias J., Giacomino S., Peñas E., Pellegrino N., Ferreyra N., Apro V., Olivera-Carrión M., Vidal-Valverde C., Assessment of the nutritional quality of raw and extruded *Pisum sativum* L. var. Laguna seeds. LWT – Food Sci. Technol., 2011, 44, 1303–1308.
  62. Fu L., Zhou C., Yao S., Yu J., Liu B., Bao J., [Plant lectins: Targeting programmed cell death pathways as antitumor agents](#). Int. J. Biochem. Cell Biol., 2011, 43, 1442–1449.
  63. Gabriel I., Lessire M., Juin H., Burstin J., Duc G., Quillien L., Thibault J.N., Leconte M., Hallouis J.M., Ganier P., Meziere N., Seve B., Variation in seed protein digestion of different pea (*Pisum sativum* L.) genotypes by cecetomized broiler chickens: 1. endogenous amino acid losses, true digestibility and in vitro hydrolysis of proteins. Livestock Sci., 2008, 113, 251–261.
  64. Ghavidel R. A., Prakash J., The impact of germination and dehulling on nutrients, antinutrients, in vitro iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT – Food Sci. Technol., 2007, 40, 1292–1299.
  65. Glencross B.D., Boujard T., Kaushik S.J., Influence of oligosaccharides on the digestibility of lupin meals when fed to rainbow trout *Oncorhynchus mykiss*. Aquaculture, 2003, 219, 703–713.
  66. Goel G., Puniya A.K., Aguilar C.F.N., Singh K., Interaction of gut microflora with tannin in feeds. Naturwissenschaften, 2005, 92 (11), 497–503.
  67. Goyoaga C., Burbano C., Cuadrado C., Romero C., Guillamón E., Varela A., Pedrosa M.M., Muzquiz M., Content and distribution of protein, sugars and inositol phosphates during the germination and seedling growth of two cultivars of *Vicia faba*. J. Food Comp. Anal., 2011, 24 (3), 391–397.
  68. Granito M., Frias J., Doblado R., Guerra M., Champ M., Vidal-Valverde C., Nutritional improvement of beans (*Phaseolus vulgaris*) by natural fermentation. Eur. Food Res. Technol., 2002, 214, 226–231.
  69. Guillamon E., Pedrosa M.M., Burbano C., Cuadrado C., de Cortes Sanchez M., Muzquiz M., The trypsin inhibitors present in seed different grain legume species and cultivar. Food Chem., 2008, 107 (1), 68–74.
  70. Guimarães-Beelen P.M., Teresinha Berchielli T., Beelen R., Araújo Filho J., de Oliveira S.G., Characterization of condensed tannins from native legumes of the Brazilian northeastern semi-arid. Sci. Agric. (Piracicaba, Braz.), 2006, 63, 522–528.
  71. Gulewicz K., Method of lupin seeds debittering 1991a. Poland, patent No 152748 (in Polish).
  72. Gulewicz K., Method of lupin seeds debittering 1991b. Poland, patent No 153195 (in Polish).
  73. Gulewicz K., Studies on complex utilization of protein and other components from bitter lupin seeds. 1988, in: Habitation dissertation (ed. K. Gulewicz) Polish Academy of Sciences Poznań, Poland (in Polish).
  74. Gulewicz K., Lupin oligosaccharides in diet – arduous or healthy factor? Procc. Polish Lupin Con. 1998. Przysiek near Toruń Proc. National Scientific Seminar “Lupin in the ecological agriculture” Przysiek k. Torunia 23.IX.1998. pp.11–23 (in Polish).
  75. Gulewicz K., Pilarski R., Legume  $\alpha$ -galactosides and their biological activity. In Proc. 11<sup>th</sup> Inter. Lupin Conference “Mexico, where old and new word lupins meet”. Mexico, 4–9 May 2005. pp.198–205.
  76. Gulewicz P., Ciesiolka D., Frias J., Vidal-Valverde C., Freinagel S., Trojanowska K., Gulewicz K., Simple method of isolation and purification of alpha-galactosides from legumes. J. Agric. Food Chem., 2000, 48, 3120–3123.
  77. Gulewicz P., Szymaniec S., Bubak B., Frias J., Vidal-Valverde C., Trojanowska K., Gulewicz K., Biological activity of alpha galactoside preparations from *Lupinus angustifolius* L. and *Pisum sativum* L. seeds. J. Agric. Food Chem., 2002, 50, 384–389.
  78. Gulewicz P., Martinez-Villaluenga C., Frias J., Ciesiolka D., Gulewicz K., Vidal-Valverde C., Effect of germination on the protein fraction composition of different lupin seeds. Food Chem., 2008, 107, 830–844.
  79. Gupta P., Dhawan K., Malhotra S.P., Singh R., Purification and characterization of trypsin inhibitor from seeds of faba bean (*Vicia faba* L.). Acta Physiol. Plantarum, 2000, 22, 433–438.
  80. Gurfinkel D. M., Rao A.V., Determination of saponins in legumes by direct densitometry J. Agric. Food Chem., 2002, 50(3), 426–430.



81. Gutierrez N., Avila C.M., Duc G., Marget P., Suso M.J., Moreno M.T., Torres A.M., CAPs markers to assist selection for low vicine and convicine contents in faba bean (*Vicia faba* L.). *Theor. Appl. Genetics*, 2006, 114, 59–66.
82. Han I.H., Baik B.K., Oligosaccharide content and composition of legumes and their reduction by soaking, cooking, ultrasound, and high hydrostatic pressure. *Cereal Chem.*, 2006, 83(4), 428–433.
83. Hassanpour S., Maherisis N., Eshratkhah B., Baghbani Mehmendar F., Plants and secondary metabolites (Tannins): A Review. *Inter. J. Forest Soil Erosion (IJFSE)*, 2011, 1(1).
84. Hassanpour S., Baghbani Mehmendar F., Anthelmintic effects of *Acacia mearnsii* (wattle tannin) in small ruminants; a review. *J. Comp. Clin. Path Res.*, 2012, 1(1), 1–8.
85. Hurrell R.F., Reddy M.B., Juillerat M.A., Cook J.D., Degradation of phytic acid in cereal porridges improves iron absorption by human subjects. *Am. J. Clin. Nutr.*, 2003, 77, 1213–1219.
86. Jimenez-Martinez C., Mora-Escobedo R., Cardador Martínez A., Muzquiz M., Martin Pedrosa M., Dávila-Ortiz G., Effect of aqueous, acid, and alkaline thermal treatments on antinutritional factors content and protein quality in *Lupinus campestris* seed flour. *J. Agric. Food Chem.*, 2010, 58 (3), 1741–1745.
87. Joanitti G.A., Azevedo R.B., Freitas S.M., Apoptosis and lysosome membrane permeabilization induction on breast cancer cells by an anticarcinogenic Bowman-Birk protease inhibitor from *Vigna unguiculata* seeds. *Cancer Lett.*, 2010, 293, 73–81.
88. Kadlec P., Bjerregaard C.H., Gulewicz K., Horbowicz M., Jones A., Knita P., Kratchanov Ch., Kratchanova M., Lewandowicz G., Soral-Smietana M., Sorensen H., Urban J., Carbohydrate chemistry. 2001, in: *Carbohydrates in legume seeds. improving nutritional quality and agronomic characteristics.* (ed. C.L. Hedley), CABI Publishing New York, pp.15–60.
89. Kaplan H., Hutkins R.W., Fermentation of fructooligosaccharides by lactic acid bacteria and bifidobacteria. *Appl. Environ. Microbiol.*, 2000, 66, 2682–2684.
90. Khattab R.Y., Arntfield S.D., Nutritional quality of legume seeds as affected by some physical treatments. 2. Antinutritional factors. *LWT – Food Sci. Tech.*, 2009, 42, 1113–1118.
91. Kobayashi M., Nagatani Y., Magishi N., Tokuriki N., Nakata Y., Tsukiyama R., Imai H., Suzuki M., Saito M., Tsuji K. Promotive effect of Shoyu polysaccharides from soy sauce on iron absorption in animals and humans. *Int. J. Mol. Med.*, 2006, 18, 1159–1163.
92. Kovalchuk N.V., Dynamic of lectin activity during bean seed *Phaseolus vulgaris* L. germination. *Ukrain'skyi Biokhimichniy Zhurnal*, 2006, 78, 130–134.
93. Kozłowska H., Aranda P., Dostalova J., Frias J., Lopez-Jurado M., Pokorny J., Urbano G., Vidal-Valverde C., Zduńczyk Z., Nutrition. 2001, in: *Carbohydrates in legume seeds. Improving nutritional quality and agronomic characteristics.* (ed. C.L. Hedley), CABI Publishing New York, pp. 61–88.
94. Kozłowska H., Honke J., Sadowaska J., Frias J., Vidal-Valverde C., Natural fermentation of lentils. Influence of time, concentration and temperature on the kinetics of hydrolysis of inositol phosphates. *J. Sci. Food Agric.*, 1996, 71, 367–375.
95. Krishnamurthy P., Tsukamoto C., Yang S.H., Lee J.D., Chung G., An improved method to resolve plant saponins and sugars by TLC. *Chromatographia*, 2012, 75, 1445–1449.
96. Kumar V., Sinha A.K., Makkar H.P.S., Becker K., Dietary roles of phytate and phytase in human nutrition: A review. *Food Chem.*, 2010, 120, 945–959.
97. Kuo Y.H., Rozan P., Lambein F., Frias J., Vidal-Valverde C., Effect of different germination conditions on the content of free protein and non-protein amino acids of commercial legumes. *Food Chem.*, 2004, 86, 537–545.
98. Liang J., Han B.Z., Nout M.J.R., Hamer R.J., Effects of soaking, germination and fermentation on phytic acid, total and in vitro soluble zinc in brown rice. *Food Chem.*, 2008, 110, 821–828.
99. Liebisch H.W., Schutte H.R., Lysine-derived alkaloids. 1985, in: *Biochemistry of alkaloids.* (eds. K. Montes, H.R. Schutte, M. Luckner). VEB Deusther Verlag der Wissenschaften Berlin pp. 150–157.
100. Liener I.E., Plant Lectin: Properties, nutritional significance and function. 1997, in: *Antinutrients and phytochemicals in food* (ed. F. Shahidi). American Chemical Society Washington, D.C. pp. 31–43.
101. Lonnerdal B., Sandberg A.S., Sandstorm B., Inhibitory effects of phytic acid and other inositol phosphates on zinc and calcium absorption in suckling rats. *J. Nutr.*, 1989, 119 (2), 211–214.
102. Lopez- Amorós M.L., Hernandez T., Estrella I., Effect of germination on legume phenolic compounds and their antioxidant activity. *J. Food Comp. Anal.*, 2006, 19, 277–283.
103. Marquardt R.R., Ward A.T., Evans L.E., Comparative properties of tannin-free and tannin-containing cultivars of faba beans (*Vicia faba*). *Can. J. Plant Sci.*, 1978, 58, 753–760.
104. Martin-Cabrejas M.A., Díaz M.F., Aguilera Y., Benítez V., Molá E., Esteban R.M., Influence of germination on the soluble carbohydrates and dietary fibre fractions in non-conventional legumes. *Food Chem.*, 2008, 107, 1047–1052.
105. Martinez-Villaluenga C., Frias J., Vidal-Valverde C., Raffinose family oligosaccharides and sucrose contents in 13 Spanish lupin cultivars. *Food Chem.*, 2005, 91(4), 645–649.
106. Martinez-Villaluenga C., Frias J., Vidal-Valverde C., Functional lupin seeds (*Lupinus albus* L. and *Lupinus luteus* L.) after extraction of  $\alpha$ -galactosides. *Food Chem.*, 2006a, 98, 291–299.
107. Martínez-Villaluenga C., Kuo Y-H., Lambein F., Frias J., Vidal-Valverde C., Kinetics of free protein amino acids, free non-protein amino acids and trigonelline in soybean (*Glycine max* L.) and lupin (*Lupinus angustifolius* L.) sprouts. *Eur. Food Res. Technol.*, 2006b, 224, 177–186.
108. Martinez-Villaluenga C., Frias J., Vidal-Valverde C., Alpha-galactosides: antinutritional factors or functional ingredients? *Crit. Rev. Food Sci. Nutr.*, 2008a, 48, 301–316.
109. Martinez-Villaluenga C., Gulewicz P., Frias J., Gulewicz K., Vidal-Valverde C., Assessment of protein fractions of three cultivars of *Pisum sativum* L.: effect of germination. *Eur. Food Res. Technol.*, 2008b, 226, 1465–1478.
110. Muzquiz M., Ridout C.L., Price K.R., Fenwick G.R., The saponin content and composition of sweet and bitter lupin seeds. *J. Sci. Food Agric.*, 1993, 63(1), 47–52.
111. Muzquiz M., Burbano C., Pedrosa M.M., Folkman W., Gulewicz K., Lupins as a potential source of raffinose family oligosaccharides: Preparative method for their isolation and purification. *Ind. Crops Prod.*, 1999, 9(3), 183–188.
112. Muzquiz M., Varela A., Burbano C., Cuadrado C., Guillamon E., Pedrosa M.M., Bioactive compounds in legumes: pro-nutritive and antinutritive actions. Implications for nutrition and health. *Phytochem. Rev.*, 2012, 11, SI, 227–244.
113. Oakenfull D., Saponins in food – a review. *Food Chem.*, 1981, 7 (1), 19–31.

114. Obendorf R.L., Górecki R.J., Soluble carbohydrates in legume seeds. *Seed Sci. Res.*, 2012, 22, 219–242.
115. Oboh H.A., Muzquiz M., Burbano C., Cuadrado C., Pedrosa M.M., Ayet G., Osagie A.U., Effect of soaking, cooking and germination on the oligosaccharide content of selected Nigerian legume seeds. *Plant Food Hum. Nutr.*, 2000, 55, 97–110.
116. Oomah B.D., Caspar F., Malcolmson L.J., Bellido A.-S., Phenolics and antioxidant activity of lentil and pea hulls. *Food Res. Int.*, 2011, 44, 436–441.
117. Page D., Quillien L., Duc G., Trypsin inhibitory measurement: Simplification of the standard procedure used for pea seed. *J. Crop Sci.*, 2000, 40, 1482–1485.
118. Peñas E., Gómez R., Frias J., Vidal-Valverde C., Effects of combined treatments of high pressure, temperature and antimicrobial products on germination of mung bean seeds and microbial quality of sprouts. *Food Contr.*, 2010, 21, 82–88.
119. Peñas E., Gómez R., Frias J., Baeza M.L., Vidal-Valverde C., High hydrostatic pressure effects on immunoreactivity and nutritional quality of soybean products. *Food Chem.*, 2011, 125, 423–429.
120. Peterbauer T., Lahuta L.B., Blochl A., Mucha J., Jones D.A., Hedley C.L., Górecki R.J., Richter A., Analysis of the raffinose family oligosaccharide pathway in pea seeds with contrasting carbohydrate composition. *Plant Physiol.*, 2001, 127, 1764–1772.
121. Petterson D.S., Ellis Z.L., Harris J., Spadek Z.E., Acute toxicity of the major alkaloids of cultivated *Lupinus angustifolius* seed to rats. *J. Appl. Toxicol.*, 1987, 7(1), 51–53.
122. Plahar W.A., Annan N.T., Nti C.A., Cultivar and processing effects on the pasting characteristics, tannin content and protein quality and digestibility of cowpea (*Vigna unguiculata*). *Plant Foods Hum. Nutr.*, 1997, 51(4), 343–356.
123. Pleszczyńska M., Szczodrak J., Tannins and their enzymatic degradation. *Biotechnologia*, 2005, 68, 152–165 (in Polish; English abstract).
124. Prodanov M., Sierra I., Vidal-Valverde C., Effect of germination on the thiamin, riboflavin and niacin contents in legumes. *Z. Lebensm. Unters. Forsch.*, 1997, 205, 48–52.
125. Pusztai A., Grant G., Assessment of lectin inactivation by heat and digestion. 1998, in: *Lectin Methods and Protocols* (eds. J.M. Rhodes, J.D. Milton), Humana Press Inc; Totowa, NJ, USA. pp. 505–513.
126. Rada V., Bartonová J., Vlková E., Specific growth rate of Bifidobacteria cultured on different sugars. *Folia Microb.*, 2002, 47, 477–480.
127. Rasha M.K., Abou-Arab E.A., Gibriel A.Y., Rasmy N.M.H., Abu-Salem F. M., Effect of legume processing treatments individually or in combination on their phytic acid content. *African J. Food Sci. Technol.*, 2011, 2(2), 036–046.
128. Reddy N.R., Pierson M.D., Sathe S.K., Salunkhe D.K., Dry bean tannins: A review of nutritional implications. *J. Am. Oil Chem. Soc.*, 1985, 62, 541–549.
129. Rodriguez C., Frias J., Vidal-Valverde C., Hernandez A., Total chemically available (free and intrachain) lysine and furosine in pea, bean, and lentil sprouts. *J. Agric. Food Chem.*, 2007, 55, 10275–10280.
130. Safavi F., Rostami A., Role of serine proteases in inflammation: Bowman-Birk protease inhibitor (BBI) as a potential therapy for autoimmune diseases. *Exp. Mol. Pat.*, 2012, 93(3), 428–433.
131. Saghai Maroof M.A., Glover N.M., Biyashev R.M., Buss G.R., Grabau E.A., Genetic basis of the low-phytate trait in the soybean line CX1834. *Crop Sci.*, 2009, 49, 69–76.
132. Sammour R.H.A., Isolation and characterization of 4 isoinhibitors from cowpea (*Vigna angularis*) walp seeds. *Tur. J. Biol.*, 2005, 30, 207–215.
133. Sandstrom B., Sandberg A.S., Inhibitory effects of isolated inositol phosphates on zinc absorption in humans. *J. Trace Elem. Electrol. Health Dis.*, 1992, 6, 99–103.
134. Sangronis E., Machado C.J., Influence of germination on the nutritional quality of *Phaseolus vulgaris* and *Cajanus cajan*. *LWT – Food Sci. Technol.*, 2007, 40, 6–12.
135. Sanz M.A., Blázquez I., Sierra I., Medrano M.A., Frias J., Vidal-Valverde C., Hernández A., Nutritional evaluation of ethanol-extracted lentil flours. *J. Agric. Food Chem.*, 2001, 49(4), 1854–1860.
136. Sendra E., Fayos P., Lario Y., Fernández-López J., Sayas-Barberá E., Pérez-Alvarez J., Incorporation of citrus fibers in fermented milk containing probiotic bacteria. *Food Microbiol.*, 2008, 25, 13–21.
137. Shi J., Arunasalam K., Yeung D., Kakuda Y., Mittal G., Jiang Y., Saponins from edible legumes: Chemistry processing and health benefits. *J. Med. Food.*, 2004, 7, 67–78.
138. Singh U., Cooking quality of pulses. *J. Food Sci. Technol.*, 1999, 36, 1–14.
139. Singh U., Jambunatham R., Studies on desi and kabuli chickpea (*Cicer arietinum* L.) cultivars – levels of protease inhibitors, levels of polyphenolic compounds and in vitro digestibility. *J. Food Sci.*, 1981, 46, 1364–1367.
140. Song Y.S., Pérez V.G., Pettigrew J.E., Martinez-Villaluenga C., Gonzalez de Mejia E., Fermentation of soybean meal and its inclusion in diets for newly weaned pigs reduced diarrhea and measures of immunoreactivity in the plasma. *Anim. Feed Sci. Technol.*, 2010, 159, 41–49.
141. Steer T.E., Gibson G.R., The microbiology of phytic acid metabolism by gut bacteria and relevance for bowel cancer. *Int. J. Food Sci. Technol.*, 2002, 37, 783–790.
142. Svanberg U., Lorri W., Fermentation and nutrient availability. *Food Contr.*, 1997, 8, 319–327.
143. Sze L.L., Tzi B.N., Lectins: production and practical applications. *Appl. Microbiol. Biotechnol.*, 2011, 89(1), 45–55.
144. Świeca M., Baraniak B., Gawlik-Dziki U., In vitro digestibility and starch content, predicted glycemic index and potential in vitro antidiabetic effect of lentil sprouts obtained by different germination techniques. *Food Chem.*, 2013, 138, 1414–1420.
145. Tabera J., Frias J., Estrella I., Villa R., Vidal-Valverde C., Natural fermentation of lentils. Influence of time, concentration and temperature on protein content, trypsin inhibitor activity and phenolic compound content. *Z. Lebensm. Unters. Forsch.*, 1995, 201, 587–591.
146. Tan N.H., Eunice Lowe S.H., Iskandar M., The extractability of winged bean (*Phosopocarpus tetragonolobus*) seed trypsin inhibitors. *J. Sci. Food Agric.*, 1982, 33, 1327–1330.
147. Tandon M., Siddique R.A., Ambwani T., Role of bypass proteins in ruminant production. *Dairy Planner*, 2008, 4(10), 11–14.
148. Thavarajah D., Thavarajah P., See C.T., Vandenberg A., Phytic acid and Fe and Zn concentration in lentil (*Lens culinaris* L.) seeds is influenced by temperature during seed filling period. *Food Chem.*, 2010, 122, 254–259.

149. Tomomatsu H., Health effect of oligosaccharides. *Food Technol.*, 1994, 48, 61–65.
150. Torres A., Frias J., Granito M., Vidal-Valverde C., Fermented pigeon pea (*C. cajan*) ingredients in pasta products. *J. Agric. Food Chem.*, 2006, 54, 6685–6691.
151. Torres A., Frias J., Granito M., Vidal-Valverde C., Título: Germinated *Cajanus cajan* seeds as ingredients in pasta products. Chemical, biological and sensory evaluation. *Food Chem.*, 2007, 101, 202–211.
152. Trafalska E., Grzybowski A., Probiotics and prebiotics in prevention of chronic civilization diseases. *New Med.*, 2006, 1, 3–6.
153. Trindade M.I., Abratt V.R., Reid S.J., Induction of sucrose utilization genes from *Bifidobacterium lactis* by sucrose and raffinose. *Appl. Environ. Microbiol.*, 2003, 69, 24–32.
154. Uauy R., Gattas V., Yaneze E., Sweet lupins in human nutrition. *World Rev. Nutr. Diet.*, 1995, 77, 75–88.
155. Urbano G., Aranda P., Gomez-Villalva E., Frejnagel S., Porres J.M., Frias J., Vidal-Valverde C., Lopez-Jurado M., Nutritional evaluation of pea (*Pisum sativum* L.) protein diets after mild hydrothermal treatment and with and without added phytase. *J. Agric. Food Chem.*, 2003, 51, 2415–2420.
156. Urbano G., López-Jurado M., Frejnagel S., Gómez-Villalva E., Porres J.M., Frias J., Vidal-Valverde C., Aranda P., Nutritional assessment of raw and germinated pea (*Pisum sativum* L.) protein and carbohydrate by in vitro and in vivo techniques. *Nutrition*, 2005, 21, 230–239.
157. Urbano G., Lopez-Jurado M., Hernandez J., Fernandez M., Moreu M.C., Frias J., Diaz-Pollan C., Prodanov M., Vidal-Valverde C., Nutritional assessment of raw, heated, and germinated lentils. *J. Agric. Food Chem.*, 1995, 43, 1871–1877.
158. van Loo J., Cummings J., Delzenne N., Englyst H., Franck A., Hopkins M., Kok N., MacFarlane G., Newton D., Quigley M., Roberfroid M., van Vliet T., van den Heuvel E., Functional food properties of non digestible oligosaccharides: a consensus report from ENDO project (DGXII AIRII-CT94–1095). *Brit. J. Nutr.*, 1999, 81, 121–132.
159. Van Rhijn P., Fijishige N.A., Lim P.O., Hirsch A.M., Sugar binding activity of pea lectin enhances heterologous infection of transgenic alfalfa plants by *Rhizobium leguminosarum* biovar viciae. *Plant Physiol.*, 2001, 126, 133–144.
160. Vandenborre G., Smaghe G., Van Damme E.J., Plant lectins as defense proteins against phytophagous insects. *Phytochemistry*, 2011, 72, 1538–1550.
161. Vasconcelos I.M., Maia F.M.M., Farias, D.F., Campello C.C., Carvalho A.F.U., de Azevedo Moreira R., de Oliveira, J.T.A., Protein fractions, amino acid composition and antinutritional constituents of high-yielding cowpea cultivars. *J. Food Comp. Anal.*, 2010, 23, 54–60.
162. Vasconcelos I.M., Oliveira J.T., Antinutritional properties of plant lectins. *Toxicon*, 2004, 44(4), 385–40.
163. Vidal-Valverde C., Frias J., Estrella I., Gorospe M.J., Ruiz R., Bacon J., Effect of processing on some antinutritional factors of lentil. *J. Agric. Food Chem.*, 1994, 42, 2291–2296.
164. Vidal-Valverde C., Frias J., Sotomayor C., Diaz-Pollan C., Fernandez M., Urbano G., Nutrients and antinutritional factors in faba beans as affected by processing. *Z. Lebensm. Unters. Forsch. A.*, 1998, 207, 140–145.
165. Vidal-Valverde C., Frias J., Sierra I., Blazquez I., Lambein F., Kuo Y.H., New functional legume food by germination. Effect on the nutritive value of beans, lentils and peas. *Eur. Food Res. Technol.*, 2002, 215, 472–477.
166. Vidal-Valverde C., Frias J., Hernandez A., Martín-Alvarez P.J., Sierra I., Rodríguez R., Blazquez I., Vicente G., Assessment of nutritional compounds and antinutritional factors in pea (*Pisum sativum*). *J. Sci. Food Agric.*, 2003, 83, 298–306.
167. Waghorn G., Beneficial and detrimental effects of dietary condensed tannins for sustainable sheep and goat production—Progress and challenges. *Animal Feed Sci. Technol.*, 2008, 147, SI, 116–139.
168. Wang H., Provan G.J., Helliwell K., Tea flavonoids: their functions, utilisation and analysis. *Trends Food Sci. Technol.*, 2000, 11, SI, 152–160.
169. Wang N., Hatcher D.W., Gawalko E.J., Effect of variety and processing on nutrients and certain anti-nutrients in field peas (*Pisum sativum*). *Food Chem.*, 2008a, 111, 132–138.
170. Wang C.-H., Lai P., Chen M.-E., Chen H.-L., Antioxidative capacity produced by *Bifidobacterium*- and *Lactobacillus acidophilus* – mediated fermentations of konjac glucomannan and glucomannan oligosaccharides. *J. Sci. Food Agri.*, 2008b, 88, 1294–1300.
171. Wang N., Hatcher D.W., Toews R., Gawalko E.J., Influence of cooking and dehulling on nutritional composition of several varieties of lentils (*Lens culinaris*). *Food Sci. Technol.*, 2009, 42, 842–848.
172. Wati R.K., Theppakorn T., Rawdkuen S., Extraction of trypsin inhibitor from three legume seeds of the Royal Project Foundation. *As. J. Food Ag-Ind.*, 2009, 2(03), 245–254.
173. Wilska-Jeszka J., Polyphenols, glucosinolanes and other pro-nutrients and antinutrients compounds. 2007, *in: Chemistry of food. Ingredients of food.* (ed. Z.E. Sikorski). WN-T, Warszawa, pp. 206–226 (in Polish).
174. Winiarska-Mieczan A., Bowman-Birk trypsin inhibitors: their structure and value in human and animal feeding. *Medycyna Wet.*, 2007, 63(3), 276–281 (in Polish; English abstract).
175. Wink M., Chemical ecology of alkaloids. 1998, *in: Alkaloids: biochemistry, ecology and medicinal application* (ed. M.F. Roberts, M. Wink). Plenum Press, New York.
176. Wyciór A., Kostyra H., Kuśmierczyk M., Food lectins. *Zywn.-Nauk. Technol. Jakość*, 2008, 6, 16 – 24 (in Polish).
177. Yamasaki, Y., Satomi, S., Murai, N., Tsuzuki, S., Fushiki, T., Inhibition of membrane-type serine protease 1/matriptase by natural and synthetic protease inhibitors. *J. Nutr. Sci. Vitaminol. (Tokyo)*, 2003, 49, 27–32.
178. Zduńczyk Z., Jankowski J., Juskiewicz J., Słominski B.A., Dietary content and gastrointestinal function of soybean oligosaccharides in monogastric animals. 2010, *in: Soybean – biochemistry, chemistry and physiology* (ed. Tzi Bun Ng). InTech, Rijeka, Croatia, Chapter 29, pp. 523–540.
179. Zheng R., Yang L., Zhou X., Zhu C., Shu X., Wu X., Li H., Wang L., Bo J., Effect of soybean oligosaccharides in immunity and TLR2--NF-κB signal pathway response for weaning pigs. *J. Food Agri. Environ.*, 2012, 10, 273–279.

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