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Bioactive compounds in legumes: pronutritive and antinutritive actions. Implications for nutrition and health

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Abstract Legume seeds are employed as a protein source for animal and human nutrition not only for their nutritional value (high in protein, lipids and dietary fibre), but also their adaptability to marginal soils and climates. Human consumption of legumes has been increased in recent years, being regarded as beneficial food ingredients. Legume seeds contain a great number of compounds which qualify as bioactive compounds with significant potentials benefits to human health. These compounds vary considerably in their biochemistry and they can be proteins, glycosides, tannins, saponins, alkaloids, etc. Hence, methods for their extraction, determination and quantification are specific of each compound. They do not appear equally distributed in all legumes, and their physiological effects are diverse. Some of these compounds are important in plant defence mechanisms against predators or environmental conditions. Others are reserve compounds, accumulated in seeds as energy stores in readiness for germination. Processing generally improves the nutrient profile of legume seed by increasing in vitro digestibility of proteins and carbohydrates and at the same time there are reductions in some antinutritional compounds. Most antinutritional factors are heat-labile, such as protease inhibitors and lectins, so thermal treatment would remove any potential negative effects from consumption. On the other hand tannins, saponins and phytic acid are heat stable but can be reduced by dehulling, soaking, germination and/or fermentation. New directions in bioactive compounds research in the last decade have led to major developments in our understanding of their role in nutrition. The scientific interest in these compounds is now also turning to studies of their possible useful and beneficial applications as gut, metabolic and hormonal regulators and as probiotic/prebiotic agents.

Keywords Antinutrients · Phytochemicals · Processing · Pulses

Introduction

There has been an increased interest in nutrition recently, not only within the scientific community but also from wider society. Nowadays, the question is not simply to eat for survival but to be informed of what we eat to enjoy the best quality of life for longer. Diet is now recognized as important, not only for nutrition, but for the prevention and treatment of diseases, especially when these diseases are caused by insufficient, excessive or unbalanced dietary intake (Dillard and German 2000; WHO 2003).

At present, one of the most controversial subjects of discussion is the establishment of an optimum human

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diet. Dietary habits are very different in each part of the world, as are the types of foods consumed. A balanced diet must contain all the necessary foods to maintain optimum nutrition. The diet must supply:

(1) Sufficient nutrient energy (calories) to carry out metabolic and physical processes.
(2) Nutrients with rheological properties and regulatory functions (Muzquiz et al. 2003).

It is very important that nutrients are balanced within the diet. The FAO-WHO Group of Experts (Nishida et al. 2004) established the following dietary recommendations: protein must not exceed 15 % of total calories, carbohydrates 60 % (3 % as fibre) and no more than 25 % lipids, comprising 75 % plant foods and 25 % derived from animal foods.

Numerous epidemiological studies have shown that a plant-based diet can reduce the risk of many chronic diseases (Dillard and German 2000; Rochfort and Panozzo 2007). The plant kingdom satisfies an important part of human diets and, depending on plant species, different parts of the plant are used: roots, tubers, stems, leaves, flowers, fruits and seeds. The legume family is one of the largest in terms of species number (Duranti 2006).

The presence of both protein and starch in adequate proportions, along with fibre, vitamins and microelements, has made the legumes a focus of justified nutritional interest (Leterme 2002). The value of pulses can be enhanced by physically fractionating the grain into basic constituents such as protein, starch and fibre and using these products to supplement other food ingredients to enhance the nutritive value of food (Rochfort and Panozzo 2007). Nevertheless, when plant foods are consumed they are often associated with a series of compounds, known as antinutrients, which generally interfere with the assimilation of some nutrients. In some cases these can be toxic or cause undesirable physiological effects (for example, flatulence). However, there is now an increased awareness of the health-associated value of pulses. Recent epidemiological studies have demonstrated that many antinutrients may be beneficial, when consumed on a regular basis, in the prevention of diseases like cancer and coronary diseases (Champ 2002).

There is a need to increase the knowledge base for pulses by understanding more of the functional and bioactive properties of pulse grain (Hill 2004). Legume seeds contain a great number of bioactive compounds that vary considerably in their biochemistry. They can be proteins (protease inhibitors, α-amylases, lectins), glycosides (α-galactosides, vicine and convicine), tannins, saponins or alkaloids (Muzquiz 2000). Hence, methods for their extraction, determination and quantification are very specific (Goyoaga et al. 2008, 2011; Pedrosa et al. 2012). They do not appear in all plants, and their physiological effects are diverse (Fig. 1). For example, pyrimidine glycosides vicine and convicine present in *Vicia faba* are the causative agents of favism; the non-protein amino acid β-N-oxalyl-L-α,β-diaminopropionic acid (β-ODAP) contained in the seeds of *Lathyrus* spp. can cause lathyrism, also known as neurolathyrism. Other bioactive compounds appear in most legumes such as the α-galactosides that produce flatulence.

Some of these bioactive compounds are important in plant defence mechanisms against predators or environmental conditions. Others are reserve compounds, accumulated in seeds as energy stores in readiness for germination (Roberts and Wink 1998).

The enormous importance of plant foods is now recognized all over the world, especially in the European Union, where the impact of plant foods on human health and the increased appreciation of the Mediterranean diet, make all these compounds more significant (Lasheras et al. 2000; Sofi et al. 2008; Aranceta et al. 2009; Elmafda and Freisling 2009; European Commission Communication 2010). The United Nations Educational, Scientific and Cultural Organization (UNESCO) inscribed the Mediterranean Diet in 2010 on the Representative List of the Intangible Cultural Heritage of Humanity (UNESCO 2010).

Existing data shows that the balance between deleterious and beneficial effects of these compounds depend on their chemical structure, concentration, time of exposure and their interaction with other dietary components (Muzquiz and Wood 2007). Thus, they can be considered as antinutrients and/or pronutrients with negative and/or positive effects on health respectively (Champ 2002; Campos-Vega et al. 2010). Hence, it is important to know not only the quantities but the types of compounds in food and how they affect the human body.

The scientific understanding of how these bioactive compounds act on an organism is an important challenge for the future. This was emphasised at the

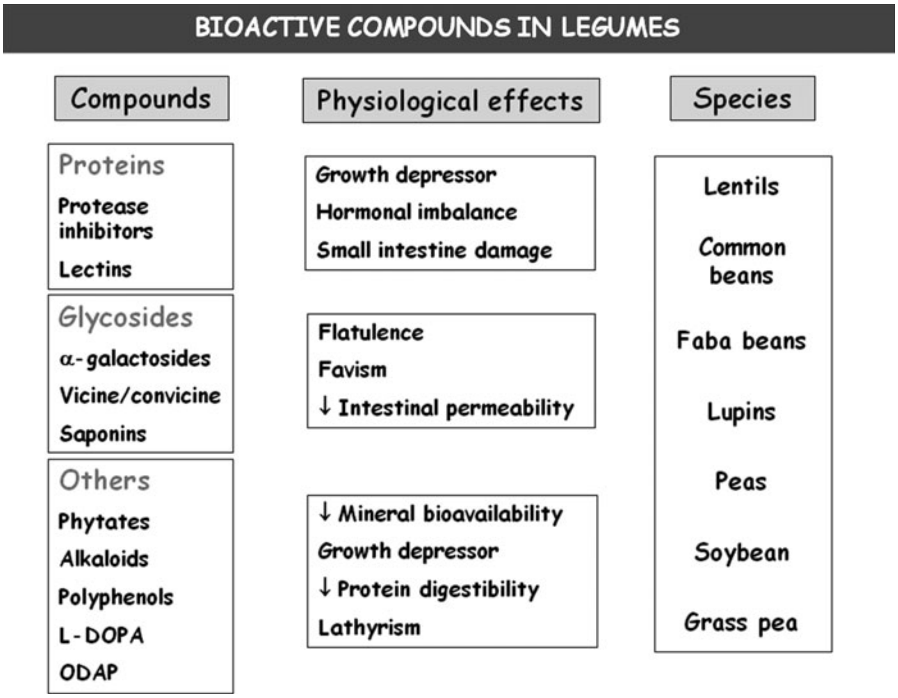

Fig. 1 Bioactive compounds in legumes

4th International Workshop on Antinutritional Factors in Legume Seeds and Oilseeds: "Recent advances of research in antinutritional factors in legume seeds and oilseeds" (2004). Special attention was paid to the beneficial and harmful effects of these compounds and to their mechanism of action in human nutrition. Thus, scientific interest is now focusing on potential beneficial applications of these compounds and the potential synergistic effects between the different classes of bioactives (Dillard and German 2000; Rochfort and Panozzo 2007).

The aim of this contribution is to review examples of some of the main bioactives found in legumes such as: oligosaccharides, phytates, protease inhibitors, α-amylase inhibitors, lectins or saponins.

Bioactive compounds

Oligosaccharides

Oligosaccharides derive their name from the Greek term *olio-*, meaning *few*, being composed of between three and ten monosaccharides joined by glycosidic bonds. The most common oligosaccharides in the plant kingdom are α-galactosides and the most ubiquitous group within these are the raffinose family of oligosaccharides (Kadlec et al. 2000). The group includes raffinose (a trisaccharide), stachyose (a tetrasaccharide), verbascose (a pentasaccharide) and ajugose (a hexasaccharide). Raffinose is present in all parts of legumes plant but accumulates in the seeds and roots during development (Muzquiz and Wood 2007). There are appreciable levels of these oligosaccharides in chickpeas, lentils, lupins, beans, peas and faba beans (0.4–16.1 % dry matter) with considerable variation between the different pulses studied (Rao and Belavady 1978; Rupérez 1998; Aranda et al. 2000; Martínez-Villaluenga et al. 2008). In Spanish legumes the concentration of raffinose range from 1.00 (*Phaseolus vulgaris* var. Palmeña) to 33.15 (*Lupinus mariae-josephi*) mg g^{-1} (Table 1). Most of the drought resistant leguminous crops contain higher raffinose contents and could contribute, in part, to their tolerance (Arora 1983). Both raffinose and stachyose have been shown to provide frost tolerance in plants (Castonguay et al. 1995). Stachyose contents range from 0.45 (*Vicia ervilia*) to 59.08 (*Lupinus albus* var. Multolupa) mg g^{-1} and verbascose is mainly present in *Pisum* and *Vicia* species. The higher amount was detected in *Pisum sativum* var. Chicarrón (49.26 mg g^{-1}), followed by *V. faba* var. Alameda (29.60 mg g^{-1}).

Table 1 α-Galactosides and ciceritol content (mg g^{-1}) in some varieties of different Spanish legumes

Sample	Raffinose	Ciceritol	Stachyose	Verbascose	Ajugose
Glycine max var. Ostrumi	9.30 ± 0.18	N.D.	40.70 ± 0.87	N.D.	N.D.
Glycine max cv. BR16	8.80 ± 0.04	N.D.	22.60 ± 0.09	2.10 ± 0.02	N.D.
Lupinus mariae-josephi	33.15 ± 3.06	10.27 ± 0.61	39.22 ± 1.85	8.39 ± 0.34	N.D.
Lupinus albus var. Marta	7.31 ± 0.05	4.04 ± 0.06	55.03 ± 0.16	11.72 ± 0.02	N.D.
Lupinus albus var. Multolupa	6.30 ± 0.06	4.01 ± 0.06	59.08 ± 0.11	11.94 ± 0.33	N.D.
Lens culinaris var. Magda	2.92 ± 0.39	12.80 ± 0.96	22.60 ± 0.99	21.9 ± 0.77	N.D.
Pisum sativum var. Chicarrón	6.58 ± 0.35	N.D.	18.92 ± 0.68	49.26 ± 1.03	N.D.
Pisum sativum var. Luna	4.20 ± 0.04	N.D.	17.54 ± 0.47	50.25 ± 0.99	N.D.
Pisum sativum var. Cartouche	5.05 ± 0.26	1.80 ± 0.09	14.59 ± 0.06	39.65 ± 0.25	N.D.
Pisum sativum var. Iceberg	5.50 ± 0.19	1.61 ± 0.15	26.30 ± 0.79	27.75 ± 0.09	N.D.
Lathyrus cicera cv. ZL-02	7.11 ± 0.11	0.93 ± 0.08	36.15 ± 0.41	N.D.	N.D.
Lathyrus cicera cv. ZL-41	6.09 ± 0.16	2.21 ± 0.13	30.73 ± 0.12	N.D.	N.D.
Cicer arietinum var. Duratón	5.59 ± 0.19	29.65 ± 0.33	18.26 ± 0.33	N.D.	N.D.
Cicer arietinum var. ELF	4.51 ± 0.11	22.83 ± 0.33	16.73 ± 0.73	N.D.	N.D.
Cicer arietinum var. Tizón	5.31 ± 0.07	26.05 ± 0.25	19.64 ± 0.19	N.D.	N.D.
Cicer arietinum var. Athenas	6.53 ± 0.87	38.3 ± 1.84	22.90 ± 0.92	N.D.	N.D.
Vicia narbonensis cv. ZU-154	4.72 ± 0.08	7.51 ± 0.35	15.89 ± 0.51	27.90 ± 0.34	N.D.
Vicia narbonensis cv. ICARDA-2470	5.47 ± 0.06	7.49 ± 0.07	17.79 ± 0.17	29.69 ± 0.44	N.D.
Vicia ervilia var. Villanueva	N.D.	10.39 ± 0.10	1.50 ± 0.27	5.81 ± 0.14	N.D.
Vicia ervilia var. Taranto	2.71 ± 0.19	10.72 ± 0.27	0.45 ± 0.08	7.83 ± 0.12	N.D.
Vicia faba var. Alameda	5.03 ± 0.12	N.D.	9.22 ± 0.38	29.60 ± 0.31	1.64 ± 0.04
Vicia faba var. Brocal	4.03 ± 0.08	N.D.	9.43 ± 0.19	28.69 ± 0.73	0.22 ± 0.01
Phaseolus vulgaris var. Planchada	2.90 ± 0.01	N.D.	29.30 ± 0.30	1.1 ± 0.01	N.D.
Phaseolus vulgaris var. Palmeña	1.00 ± 0.00	N.D.	29.30 ± 0.30	1.1 ± 0.01	N.D.

N.D. not detected

Burbano et al. (1999)

Goyoaga et al. (2011)

Muzquiz et al. (2009)

Muzquiz et al. (2011)

Pedrosa et al. (2012)

Trugo et al. (1999)

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Ph. vulgaris usually contained negligible concentrations of verbascose and ajugose was only detected in *V. faba* seeds. Ciceritol is also an α-galactoside but does not belong to the raffinose family of oligosaccharides. It is an α-D-digalactoside of pinitol and was first discovered in chickpea (hence the name) by Quemener and Brillouet (1983). These authors found that ciceritol was also present in lupin, lentil, pea and some *Vicia* species. It is thought that ciceritol was mistakenly identified as manninotriose in previous literature (Bernabe et al. 1993). Chickpea seeds contain 21.1–31.0 mg g^{-1} and 12.4–38.3 mg g^{-1} ciceritol in desi and kabuli types, respectively (Muzquiz and Wood 2007; Pedrosa et al. 2012).

The α-galactosides are well known as antinutritional factors for causing flatulence. The first information on the antinutritive effects of α-galactosides was reported back by Kuriyama and Mendel (1917). Flatulence occurs because mammals (including humans) have no α-galactosidase present in their intestinal mucosa which is required to hydrolyse these compounds (Muzquiz et al. 1999a, b). Hence, ingestion results in α-galactosides passing into the large intestine where bacteria anaerobically ferment the α-galactosides with the production of hydrogen, CO_2 and traces of methane gas (Rochfort and Panozzo 2007). These authors also reported that flatulence is sometimes accompanied by diarrhoea and abdominal pain if large quantities are consumed. Whilst raffinose and stachyose ingestion is known to cause flatulence (Fleming 1981), this is not the case for ciceritol, perhaps by easier hydrolysis of this compound due to its different structure (Quemener and Brillouet 1983; Muzquiz and Wood 2007).

The lack of digestion is also a negative for intensive monogastric livestock producers, as oligosaccharides in the diet can reduce growth performance. In addition, a high content of α-galactosides (higher than found in normal diets) has been shown to reduce the absorption capacity of the small intestine by changing the osmotic pressure in the small intestine (Wiggins 1984; Zdunczyk et al. 1998). However, intestinal digestion of α-galactosides can be improved by supplementation of diets with exogenous α-galactosidase (Kozlowska et al. 2001). Contrary to previous reports, research by Sandberg et al. (1993) showed recently that about 30 % of dietary raffinose and stachyose is degraded in the stomach and small intestine of human subjects and digested.

Although α-galactosides have little food value (are partially digested, if at all) this does not imply the absence of nutritional value. In fact, α-galactosides convey many benefits to humans and mongastric animals alike. Since they pass mostly undigested into the lower gut, α-galactosides are a constituent of dietary fibre and can act as a probiotic (Schley and Field 2002). Dietary fibre has been reported to convey many benefits. In addition, the probiotic effect is derived from metabolism of α-galactosides by gas-producing bacteria increasing the colonic population of bifidobacteria. Bifidobacteria are known to contribute positively to human health by suppressing intestinal putrefaction, reducing both constipation and diarrhoea, stimulating the immune system and increasing resistance to infection (Mitsuoka 1996; Eiwegger et al. 2004).

Phytic acid

Pulse grains are a dietary source of minerals, although their bioavailability is considered lower because of the concentration of phytate (Sandberg 2002). Phytate is formed during maturation of the plant seed and in dormant seeds represents 60–90 % of the total phosphate (Loewus 2002). Usually legume based food items contain higher amounts phytate than do cereal-based food items (Kumar et al. 2010).

Phytic acid, myo-inositol-(1,2,3,4,5,6) hexakisphosphate, and its salts represent the majority of the phosphorus in legume seeds (Urbano et al. 2000; Kumar et al. 2010). According to different authors the total inositol phosphates content (% dry matter) ranged from 0.2 to 1.9 % in *Ph. vulgaris*, from 0.15 to 2.34 % in *Lens esculenta*, from 0.4 to 1.1 % in *Cicer arietinum*, from 0.2 to 1.3 in *P. sativum* or from 0.5 to 1.1 % in *V. faba* (Campos-Vega et al. 2010). The total inositol phosphates content in some Spanish legumes has been reported to vary from 0.35 % in *L. mariae-josephi* to 2.0 % in *Glycine max* (Table 2). Inositol phosphates content can vary with genotype, climate, type of soil, and year (Burbano et al. 1999).

The myo-inositol phosphates, IP_6 and IP_5, have the worst antinutritional effects, as the smaller molecules (IP_4, IP_3, IP_2, IP_1) have a lower capacity to complex with inorganic cations. IP_4-IP_3 were present in lower concentration than IP_6-IP_5 and some legumes did not contain these smaller forms. The major inositol phosphate in legumes is IP_6 (Burbano et al. 1995); the lower amount was present in *L. mariae-josephi* and *Ph. vulgaris* var. Planchada seeds (both 0.34 % dry matter), and the higher content correspond to *G. max* var. Ostrumi (1.76 % dry matter) seeds.

Phytic acid has been considered an antinutrient as it binds with other nutrients making them inaccessible to digestion. Excessive phytic acid in the diet can have a negative effect on mineral balance because of the insoluble complexes it forms with essential minerals (Cu^{2+}, Zn^{2+}, Fe^{3+} and Ca^{2+}) which causes poor mineral bioavailability (Zhou and Erdman 1995; Urbano et al. 2000; Konietzny and Greiner 2003). Among them, bioavailability of Zn^{2+} was reported to be the most adverse effect in humans (Lopez et al. 2002). Phytic acid is able to complex with proteins also, decreasing protein solubility. Phytates therefore impact on enzyme activity and there is evidence of negative effects on key digestive enzymes including lipase, α-amylase, pepsin, trypsin and chymotrypsin (Thompson 1993; Greiner and Konietzny 1996; Urbano et al. 2000). The binding of phytic acid to these enzymes reduces nutrient digestibility. Phytic acid also appears to bind with starch through phosphate linkages (Lajolo et al. 2004).

Table 2 Inositol phosphates content (mg g^{-1}) in some varieties of different Spanish legumes

Sample	IP3	IP4	IP5	IP6	Total IP
Glycine max var. Ostrumi	0.49 ± 0.01	0.51 ± 0.02	1.53 ± 0.04	17.6 ± 0.08	20.1 ± 0.15
Glycine max cv. BR16	N.D.	0.14 ± 0.01	0.82 ± 0.02	3.82 ± 0.15	4.78 ± 0.20
Lupinus albus var. Multolupa	N.D.	0.52 ± 0.00	1.07 ± 0.05	7.54 ± 0.15	8.87 ± 0.12
Lupinus luteus	N.D.	N.D.	0.60 ± 0.00	7.17 ± 0.12	7.77 ± 0.12
Lupinus mariae-josephi	N.D.	0.17 ± 0.00	N.D.	3.42 ± 0.16	3.46 ± 0.12
Lens culinaris var. Magda	0.62 ± 0.02	1.17 ± 0.06	2.81 ± 0.13	7.63 ± 0.04	12.2 ± 0.22
Pisum sativum var. Chicarrón	0.07 ± 0.00	N.D.	0.38 ± 0.05	5.01 ± 0.21	5.46 ± 0.17
Pisum sativum var. Luna	0.06 ± 0.00	N.D.	0.38 ± 0.03	4.05 ± 0.11	4.46 ± 0.15
Pisum sativum var. Cartouche	0.06 ± 0.00	N.D.	0.44 ± 0.01	5.94 ± 0.16	6.43 ± 0.15
Pisum sativum var. Iceberg	0.07 ± 0.00	0.05 ± 0.03	0.52 ± 0.04	5.79 ± 0.10	6.43 ± 0.17
Lathyrus cicera cv. ZL-02	0.07 ± 0.01	N.D.	0.44 ± 0.01	5.27 ± 0.11	5.79 ± 0.11
Lathyrus cicera cv. ZL-41	0.10 ± 0.01	N.D.	0.49 ± 0.02	8.24 ± 0.32	8.83 ± 0.35
Cicer arietinum var. Duratón	0.08 ± 0.00	N.D.	0.46 ± 0.04	6.36 ± 0.08	6.91 ± 0.10
Cicer arietinum var. ELF	0.07 ± 0.00	N.D.	0.34 ± 0.02	5.30 ± 0.06	5.71 ± 0.07
Cicer arietinum var. Tizón	N.D.	N.D.	0.36 ± 0.02	5.73 ± 0.11	6.10 ± 0.09
Cicer arietinum var. Athenas	N.D.	0.57 ± 0.02	1.44 ± 0.06	4.01 ± 0.02	6.02 ± 0.07
Vicia narbonensis cv. ZU-154	0.06 ± 0.01	N.D.	0.34 ± 0.01	5.46 ± 0.13	5.84 ± 0.14
Vicia narbonensis cv. ICARDA-2470	0.09 ± 0.01	0.10 ± 0.00	0.43 ± 0.02	7.94 ± 0.15	8.51 ± 0.19
Vicia ervilia var. Villanueva	0.19 ± 0.02	N.D.	0.31 ± 0.02	6.21 ± 0.20	6.71 ± 0.21
Vicia ervilia var. Taranto	0.21 ± 0.00	N.D.	0.32 ± 0.06	8.11 ± 0.10	8.64 ± 0.04
Vicia faba var. Alameda	0.25 ± 0.01	0.63 ± 0.02	2.15 ± 0.04	5.54 ± 0.06	8.56 ± 0.11
Vicia faba var. Brocal	0.15 ± 0.00	0.44 ± 0.00	1.69 ± 0.03	4.21 ± 0.10	6.49 ± 0.08
Phaseolus vulgaris var. Planchada	0.04 ± 0.00	0.04 ± 0.00	0.53 ± 0.04	3.44 ± 0.24	4.00 ± 0.28
Phaseolus vulgaris var. Palmeña	N.D.	N.D.	0.49 ± 0.05	4.46 ± 0.01	4.95 ± 0.04

N.D. not detected

Goyoaga et al. (2011)

Muzquiz et al. (1999b)

Muzquiz et al. (2009)

Muzquiz et al. (2011)

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Trugo et al. (1999)

The ability of phytic acid to bind with minerals, proteins or starch, directly or indirectly, may alter solubility, functionality, digestibility and absorption of these nutrients. In addition, monogastric animals have a limited ability to hydrolyse phytates and release phosphate for absorption due to a lack of intestinal phytases (Zhou and Erdman 1995; Greiner and Konietzny 1996; Urbano et al. 2000).

However, there are some beneficial effects of phytic acid, such as reduced bioavailability, and therefore toxicity, of heavy metals (such as cadmium and lead) present in the diet (Rimbach et al. 1996; Rimbach and Pallauf 1997). Several studies have provided evidence of phytic acid displaying anti-oxidant properties in vitro (Lajolo et al. 2004). These effects are mainly mediated through its iron and copper chelating properties, although the molecular mechanisms are not fully understood. Contrary to this was research by Minihane and Rimbach (2002) who demonstrated under in vivo conditions that phytic acid did not always have a significant effect on oxidant or anti-oxidant status.

Some myo-inositol phosphates, including IP_6 from soybean, have been suggested to have beneficial health effects, such as amelioration of heart disease by controlling hypercholesterolemia and atherosclerosis, prevention of kidney stone formation and a reduced risk of colon cancer (Champ 2002; Greiner et al. 2002). IP_6 has been suggested to be responsible for the epidemiological link between high-fibre diets (rich in IP_6) and low incidence of some cancers (Campos-Vega et al. 2010). The anticarcinogenic properties of phytic acid may result, from numerous factors, including its ability to chelate metal ions (Steer and Gibson, 2002). In mice, dietary myo-inositol has been shown to be effective in preventing different cancer (Wattenberg et al. 2000; Nishino et al. 1999; Jenab and Thompson 2002).

Phytases are enzymes that are widely distributed in nature in plants, certain animal tissues and in microorganisms (Haros et al. 2007; Hill et al. 2007). They have been studied intensively in the last few years because of great interest their ability to reduce the phytate content in monogastric and human foods (Greiner and Konietzny 2006). Enzymatic degradation of phytic acid by exogenous phytase is already used in feed, particularly to improve mineral and protein utilization, simultaneously reducing excessive phosphorous accumulation in the environmental (Grases et al. 2006). Phytases do this by hydrolyzing phytic acid to a series of lower phosphate esters of myo-inositol and phosphate. IUPAC-IUBMB (the International Union of Pure and Applied Chemistry and the International Union of Biochemistry and Molecular Biology) currently acknowledges three classes of phytase enzymes, which initiate the dephosphorylation of phytic acid at different positions on the inositol ring, and produce different isomers of the lower inositol phosphates (Bohn et al. 2008). These three classes of phytase enzymes are: 3-phytase (EC 3.1.3.8), 5-phytase (EC 3.1.3.72) and 4/6-phytases (EC 3.1.3.26), indicating the susceptible phosphoester bond that is predominantly attacked by the enzyme. The pathway of dephosphorylation of myo-inositol hexakisphosphate by phytases purified from different legume seeds has been established by Maiti et al. (1974) and modified by Greiner et al. (2002).

Different structural isomers of myo-inositol phosphates can be generated during enzymatic degradation. They can have different physiological functions; hence, their identification is of great importance to exploit the full potential of naturally occurring phytases (Greiner et al. 2002).

Protein-antinutrients

The most widely studied antinutrient proteins in legumes are the enzyme inhibitors (pancreatic proteases and α-amylases) and the lectins (Lajolo et al. 2004; Pusztai et al. 2004).

Protease inhibitors

Legume seed protease inhibitors can have a major impact on seed nutritional value as they inhibit the function of digestive enzymes, such as trypsin and chymotrypsin, by competitive binding. These protease inhibitors contain no carbohydrates and belong to two different families; the Kunitz family and the Bowman-Birk family. Protease inhibitors from both families have been found in legume seeds (Domoney 1999; Lajolo and Genovese 2002; Lajolo et al. 2004; Srinivasan et al. 2005). Both families of protease inhibitors are capable of inhibiting trypsin and chymotrypsin. A large number of isoforms of the Bowman-Birk inhibitor (BBI) have been described in *G. max* and have differing properties depending on their chemical structure. Guillamón et al. (2008a) showed that trypsin inhibitor isoform varied with legume species and variety.

However, this is unlikely to be a problem in humans as protease inhibitors are heat-labile and legumes are usually cooked prior to consumption. Genetic engineering is being also used to reduce protease inhibitors (Greiner and Domoney 2004).

The trypsin inhibitors content in different Spanish legumes ranged form 5.75 TIU mg^{-1} (*P. sativum* var. Iceberg) to 83.70 TIU mg^{-1} (*G. max* var. Ostrumi); however, *G. max* var. Ostrumi did not contain chymotrypsin inhibitors. *Ph. vulgaris* var. Riñón and *Lathyrus cicera* presented the highest values (>16 CIU mg^{-1}) (Table 3).

Antinutritional effects and health benefits have been ascribed to the presence of certain amounts and types of protease inhibitors. The effect of trypsin inhibitors on animal growth is not only a consequence of inhibition of intestinal protein digestion, since the presence of inhibitors in diets consisting of free amino acids also led to decreased growth (Lajolo et al. 2004). The Kunitz and Bowman-Birk inhibitors have been found to cause an enlargement of the pancreas (hypertrophy and hyperplasia) and hypersecretion of digestive enzymes (sulphur-rich proteins) in rodents

Table 3 Trypsin inhibitors activity (TIU mg^{-1}) and chymotrypsin inhibitors activity (CIU mg^{-1}) in some varieties of different Spanish legumes

Sample	TIU mg^{-1}	CIU mg^{-1}
Glycine max var. Ostrumi	83.70 ± 6.20	N.A.
Glycine max var. Ostrumi deffated	8.57 ± 0.14	12.00 ± 0.16
Pisum sativum var. Chicarrón	9.94 ± 0.50	12.22 ± 0.75
Pisum sativum var. Luna	12.55 ± 0.07	15.75 ± 0.21
Pisum sativum var. Cartouche	9.87 ± 0.71	10.16 ± 0.28
Pisum sativum var. Iceberg	5.75 ± 0.08	8.62 ± 0.38
Lathyrus cicera cv. ZL-02	15.14 ± 0.38	16.60 ± 0.50
Lathyrus cicera cv. ZL-41	16.28 ± 0.38	20.78 ± 0.14
Cicer arietinum var. Duratón	14.51 ± 0.28	10.12 ± 0.61
Cicer arietinum var. ELF	12.60 ± 0.45	9.74 ± 0.22
Cicer arietinum var. Tizón	12.70 ± 0.35	11.38 ± 0.90
Vicia narbonensis cv. ZU-154	6.95 ± 0.01	2.19 ± 0.10
Vicia narbonensis cv. ICARDA-2470	7.01 ± 0.05	2.63 ± 0.60
Vicia ervilia var. Villanueva	6.52 ± 0.11	8.68 ± 0.57
Vicia ervilia var. Taranto	7.60 ± 0.08	8.20 ± 0.12
Phaseolus vulgaris var. Riñon	13.60 ± 0.68	17.30 ± 0.39

N.A. not analyzed

TIU means trypsin inhibitor units

CIU means chymotrypsin inhibitor units

Rubio et al. (2006)

Spanish CDTI Project (IDI-20100284)

and birds. This results in a loss of the sulphur-rich endogenous proteins that would cause growth depression, as legume seed proteins are generally deficient in the sulphur amino acids (Lajolo and Genovese 2002).

On the other hand, protease inhibitors have been linked, over the last two decades, to health promoting properties (Champ 2002) and are considered natural bioactive substances. Protease inhibitors may act as anticarcinogenic agents (Clemente et al. 2004). Bowman-Birk inhibitors have been shown to be effective in preventing or suppressing carcinogen-induced transformation in vitro and carcinogenesis in animal assays. The BBI, achieved Investigational New Drug status from the FDA in 1992 for this purpose (Kennedy 1995) and studies with humans showed no toxic effects of BBI (Amstrong et al. 2000). Use of BBI inhibited the growth and survival of human prostate cancer cells (Kennedy and Wan 2002). It also reduced the incidence and frequency of colon tumours in dimethylhydrazine-treated rats. However, this effect was not observed with autoclaved BBI, suggesting that protease inhibitor activity was necessary for anticarcinogenic activity (Kennedy et al. 2002).

α-Amylase inhibitors

Alpha-amylases (α-1,4-glucan-4-glucanohydrolases) are endo-amylases that catalyze the hydrolysis of α-D-(1,4) glycosidic linkages which occur in starch and related compounds. They play a major role in the carbohydrate metabolism of animals and humans by providing them with glucose as an energy source and as a building block for synthesis of other sugars. Among α-amylase inhibitors (αAI) found in plants, legume αAI, and especially αAI from beans, have received considerable attention (Whitaker 1988; Lajolo and Genovese 2002). Jaffe et al. (1973) screened 95 legume cultivars for αAI levels and found that lima beans (*Phaseolus lunatus*), mung beans (*Phaseolus aureus*) and horse gram (*Dolichos biflorus*) had the highest levels of inhibitory activity. Mulimani et al. (1994) determined the αAI activity of 28 varieties of chickpea and found variations ranging from 11.6 inhibitory units g^{-1} to 51.4 inhibitory units g^{-1}. Alonso et al. (1998) reported 16.8 inhibitor units g^{-1} for *P. sativum* cv. Solara and Marzo et al. (2001) found 2.26 inhibitor units g^{-1} for *Ph. vulgaris* var. Pinto. Jaffe et al. (1973) reported that the partially purified kidney bean inhibited the salivary amylase more than the pancreatic amylase. This shows that amylase inhibitors from different legume seeds may exhibit unequal activity against different enzymes.

The most probable function of αAI in the plant is in protection from predatory insects by inhibiting their digestive amylase. These αAIs have been shown to inhibit pancreatic amylases (human and porcine), human salivary amylase and insect amylase (Le Berre-Anton et al. 1997). α-Amylase inhibitors are deleterious or anti-metabolic for many predators of crop plants. The genes for both of these factors have been introduced into a number of plants and the resultant transgenic crops have a significantly increased resistance to predators (Gatehouse et al. 1994; Gatehouse 2011). Campbell et al. (2011) described the development of three genetically modified legumes (pea, chickpea and cowpea) containing a gene from the common bean (*Ph. vulgaris*) for αAI to protect peas in the field from pea weevil and the harvested grain of chickpea and cowpea from larvae of cowpea weevil, a prevalent pest that causes serious damage in many developing countries in Africa and Asia.

High dietary intakes of αAIs can cause a number of potentially deleterious alterations in the body metabolism of experimental animals. Significant enlargement of the small intestine and of the pancreas was evident in rats given αAI (Grant et al. 1998).

α-Amylase inhibitors reduce amylase activity and starch digestion in the gut when given orally to humans (Singh et al. 1982). As a result, they lower postprandial increases in circulating glucose and insulin. These inhibitors may, therefore, prove useful in treatments for obesity or diabetes mellitus.

The stability of αAI to proteolytic degradation in vivo does not seem to have been evaluated. However, the findings that orally administered αAI could greatly reduce intraluminal amylase levels and starch digestion in rats and humans suggests that nutritionally significant quantities of αAI survive passage through the small intestine in a fully functional form (Pusztai et al. 1995).

Lectins

Lectins (haemagglutinins) are glycoproteins which are able to reversibly bind to specific sugars and glycoproteins on the surface of cells in the gut wall, thereby interfering with nutrient breakdown and absorption. This reaction is manifested, in vitro, by agglutination of red blood cells from various animal species. Lectins have traditionally been measured by their haemagglutinating activity (Grant 1991). Lectins are extremely specific; different types having different interactions on toxicity, blood groups, mitogenesis, digestion and agglutination.

Two haemagglutination assay systems have been used: native rat blood cells and blood cells treated with trypsin to increase the sensitivity of the assay. *Ph. vulgaris* var. Processor and Pinto are included in each assay as standards and 1 HU (haemagglutination unit) is defined as the amount of material in the last dilution at which 50 % of the cells were agglutinated. However, when possible, ELISA based immunological methods which recognise specific antibodies are being increasingly used due to their higher specificity (Hajós et al. 1996; Muzquiz et al. 2001). Table 4 shows the lectin content of different Spanish legumes measured by both methodologies. *L. culinaris* showed the highest hemaglutinating capacity (500.0 HU g Kg^{-1}) of the analyzed legumes. Standard lectin of soybean (SBA), lentil (LcA) and beans (PHA) were used to carry out a competitive indirect ELISA. *Ph. vulgaris* var. Oracada, Curruquilla and Processor showed the highest values (>12 g kg^{-1}).

Nutritionally dietary lectins vary considerably in the nature and extent of their antinutritional effects. Lectins can be toxic; they can interfere with hormone balance and deplete nutrient reserves leading to severe growth depression and a high incidence of deaths. In short experiments, purified lectins from beans or soybeans impaired growth of rats, induced enlargement of the small intestine, caused damage to the epithelium of the small intestine, and stimulated hypertrophy and hyperplasia of the pancreas. A proximal lesion (reduction in villus cell population) with increase in enterocyte population of the jejunal villi and length, population, and crypt cell production of the jejunal and ileum crypts were observed (Zucoloto et al. 1991). Reduction in maltase and invertase activities of the intestinal mucosa and interference with glycose transportation were also reported (Figueroa et al. 1984). At higher levels, bean lectins induced depletion of body and skeletal muscle, lipid, and glycogen (Bardocz et al. 1992, 1996).

However, lectins may be beneficial by stimulating gut function, limiting tumour growth and ameliorating obesity (Pusztai et al. 2004). The inclusion of purified bean phytohemagglutinin in the diet of mice reduced tumour growth rate in a dose-dependent manner, suggesting a competition for nutrients between the gut epithelium undergoing hyperplasia and the developing tumour (Pryme et al. 1998). In obese rats, a reduction of lipid accumulation by inclusion of raw kidney bean in the diet was observed, which was related to a decrease of insulin levels caused by lectins. However, no body or muscle protein losses occurred, even at

Table 4 Lectin content measured by Haemagglutination Units and competitive indirect ELISA (g Kg^{-1}) in some varieties of different Spanish legumes

Sample	HU	Elisa lectin
Glicine max var. Ostrumi	40.00 ± 0.00	4.80 ± 0.10
Lens culinaris var. Magda	500.00 ± 1.67	8.00 ± 0.27
Phaseolus vulgaris var. Oracada	40.00 ± 0.00	12.07 ± 0.73
Phaseolus vulgaris var. Corcal	3.00 ± 0.00	1.53 ± 0.43
Phaseolus vulgaris var. Gernikesa	24.09 ± 0.50	5.50 ± 0.50
Phaseolus vulgaris var. Tolosana	17.01 ± 1.50	6.49 ± 1.50
Phaseolus vulgaris var. Almonga	0.32 ± 0.00	0.67 ± 0.00
Phaseolus vulgaris var. Curruquilla	4.00 ± 0.00	12.87 ± 0.79
Phaseolus vulgaris var. Processor	20.41 ± 0.02	25.25 ± 1.90
Pisum sativum var. Chicarrón	0.32 ± 0.00	N.A.
Pisum sativum var. Cartouche	0.24 ± 0.08	N.A.
Pisum sativum var. Luna	0.63 ± 0.00	N.A.
Pisum sativum var. Iceberg	0.32 ± 0.00	N.A.
Vicia ervilia var. Villanueva	0.48 ± 0.16	N.A.
Vicia ervilia var. Taranto	0.63 ± 0.00	N.A.
Lathyrus cicera cv. ZL-02	1.28 ± 0.00	N.A.
Lathyrus cicera cv. ZL-41	0.48 ± 0.16	N.A.

N.A. not analyzed

Pedrosa et al. (2012)

Asensio-Vegas et al. (2007)

Muzquiz et al. (1999a)

Spanish CDTI Project IDI-20100284 (2011)

high doses, as with normal rats, suggesting a possible use of lectins as therapeutic agents to treat obesity (Pusztai et al. 1998).

Saponins

Saponins derive their name from their ability to form stable, soap like, foams in aqueous solutions. They are a complex and chemically diverse group of compounds. Chemically saponins are composed of a steroidal or triterpene aglycone linked to one, two or three saccharide chains of varying size and complexity via ester and ether linkages. The complexity of the saponin structure (and thereby their diversity of biological activities) depends on the variability of the aglycone structure, the attachment position of the glycosidic moieties, and the nature of these glycosides. Aglycones are generally linked to D-galactose, L-rhamnose, D-glucose, D-xylose, D-mannose and D-glucuronic acids some of which may be acetylated (Fenwick et al. 1991). All legumes have triterpene-type saponins.

The chemical composition of soybean (*G. max*) saponins has been extensively investigated. They are triterpene saponins (known as soyasaponins) of which more than 10 types have been isolated (Shibuya et al. 2006). Saponins have been reported in many edible legumes, and they have been found in lupins, lentils and chickpeas, as well as soy, various beans and peas (Ruiz et al. 1996a; Woldemichael et al. 2003; Shi et al. 2004). Soya-sapogenol B was identified as the aglycone in saponins from different legume species (Price et al. 1987, 1988; Ayet et al. 1996; Tava et al. 1993). Ayet et al. (1996) and Burbano et al. (1999) found sapogenol-B in different Spanish legumes, with contents of 0.36 mg g^{-1} in Chickpea (Kabuli type) and lentil (var. Magda). *Ph. vulgaris* varieties always contained the highest values (from 0.44 to 2.05 mg g^{-1}) and *V. faba* the lowest (0.02 mg g^{-1}). Ruiz et al. (1996b, 1997) showed the presence of soyasaponin VI, a conjugated form of soyasaponin I, in many legumes. Soyasaponin VI may have an important physiological role in preventing lipid peroxidation of DNA and proteins by free radical attack.

Ingestion of saponin-containing plant foods by humans and animals has been associated with both deleterious and beneficial effects (Oakenfull and Sidhu 1989).

Their physiological activity appears to arise from two main causes: their powerful physical interactions, as surface-active agents, with other components of the digesta and their ability to interact with the membranes of mucosal cells. Sometimes the latter causes profound changes in the associated membrane biochemistry. These physiological interactions can inhibit nutrient uptake.

Potential benefits from consumption of saponins in foods include a reduced risk of cardiovascular disease and some cancers. Dietary saponins have been repeatedly shown to lower plasma cholesterol in animals. However, their hypocholestoremic effect in humans is more speculative. Some studies suggest that saponins may reduce cholesterol through the formation of insoluble complex with cholesterol, thus preventing absorption in the intestine. Additionally, some saponins increase the excretion of bile acids, an indirect method to decreasing cholesterol (Rochfort and Panozzo 2007). They may also have anticarcinogenic properties, as suggested by a recent rodent study in which feeding a saponin-containing diet inhibited the development of preneoplastic lesions in the colon (Koratkar and Rao 1997).

Clinical studies have suggested that these health-promoting components, saponins, affect the immune system in ways that help to protect the human body against cancers, and also lower cholesterol levels. Saponins decrease blood lipids, lower cancer risks, and lower blood glucose response. A high saponin diet can be used in the inhibition of dental caries and platelet aggregation, in the treatment of hypercalciuria in humans, and as an antidote against acute lead poisoning. In epidemiological studies, saponins have been shown to have an inverse relationship with the incidence of renal stones (Shi et al. 2004).

Processing

Processing generally improves the nutrient profile of legume seed by increasing in vitro digestibility of proteins and carbohydrates from around 40 % up to 98 % (Rehman and Shah 1998; el-Adawy 2002; Naveeda and Jamuna 2004). At the same time, there are reductions in some antinutritional compounds (Muzquiz et al. 1996; Hajós and Osagie 2004; Jiménez-Martinez et al. 2004).

Among the traditional processing techniques we can outline: (a) Non-heat processing such as imbibition, germination, dehulling or fermentation, and (b) Heat processing, such as cooking at atmospheric pressure, autoclaving or roasting (Cuadrado et al. 1996; Egounlety and Aworth 2003; Martín-Cabrejas et al. 2004; Rehman and Shah 2005; Sánchez et al. 2005). Most antinutritional factors are heat-labile, such as protease inhibitors and lectins, so cooking would remove any potential ill effects from consumption (Rochfort and Panozzo 2007). Tannins, saponins and phytic acid, on the other hand, are heat stable but can be reduced by dehulling, soaking, germination and/or fermentation.

Dehulling is a common processing step that removes the seed coat, which contains some of the antinutritional factors, such as tannins.

Imbibition is commonly done before cooking whole legume seeds, mainly to reduce cooking times (Wood and Harden 2006). However, soaking also reduces certain antinutritional factors which leach into the soaking medium, such as oligosaccharides, protease inhibitors and some tannins (Saxena et al. 2003). The amount of leaching will vary depending on the soaking medium (water, salt solution or bicarbonate solution) and on the soaking time. For example, Rao and Deosthale (1982) found that the tannin content of chickpeas reduced by almost 50 % after soaking overnight in water.

The effects of germination and cooking treatments on the nutritional composition and antinutritional factors of legumes were studied by el-Adawy (2002) and Goyoaga et al. (2011). They found that germination was more effective in reducing phytic acid, stachyose and raffinose than cooking. Greiner et al. (1998) reported a large reduction in phytate due to germination in lentils but not in chickpeas. Rao and Deosthale (1982) found germination to decrease tannin content of chickpeas by a further 10 % after soaking overnight.

Cooking (boiling, autoclaving and microwave cooking) were found to be more effective in reducing trypsin inhibitors, hemagglutinin activity, tannins and saponins than germination (el-Adawy 2002).

Fermentation significantly decreased both phytic acid and tannin contents in chickpea tempeh flour (Reyes-Moreno et al. 2004). Saponin contents of soybean were halved in fermented tempeh compared to the raw seed (Fenwick and Oakenfull 1983).

Increased demand for food products, whose nutritional and sensory quality is retained, or improved, has allowed the introduction of new food processing technologies like extrusion/cooking (extrusion HTST), microwaving and instant controlled pressure drop (DIC). The advantages of hot extrusion against other heat processing methods are improved nutritive value of the raw material. It is a versatile processing technique that can produce a variety of products of different form, texture and organoleptic characters, tailored to market demand, from similar raw materials. It can also reduce thermolabile factors such as lectins and trypsin inhibitors and increase starch and protein digestibility (Urdaneta et al. 2003). Alonso et al. (2001) extruded pea (*P. sativum*) and kidney bean (*Ph. vulgaris*) meals, which reduced tannin, oligosaccharide, starch, non starch polysaccharides (NSP) and lectin contents, and increased mineral availability.

There are contradictory results concerning the effect of microwaving on nutrients; some suggest that increased nutritive value is only obtained when low power is used for a short exposure time. It seems that power is the most important parameter affecting the results, but there are few studies on the optimisation of the physical parameters involved. Studies on soyabean, using dry or soaked seed, and using different time and temperature conditions show a reduction in trypsin inhibitor and lectin content with improved starch digestibility, without any significant effect on nutritional quality (Rajkó et al. 1997; Mahungu et al. 1999). Autoclaving was found to be more effective in reducing lupin allergenicity than extrusion, boiling, or microwaving (Alvarez-Alvarez et al. 2005).

DIC treatment is a new and highly controlled process used in food technology that combines steam pressure (up to 8 bar) with heat (up to 170 °C) for a short time (up to 3 min), DIC treatment of some bioactive compounds in lupin and soybean seeds considerably reduced these components without affecting the total protein or lipid contents (Haddad et al. 2006, 2007; Haddad and Allaf 2007; Pedrosa et al. 2012).

Previous studies have demonstrated that lupin allergenic proteins are relatively heat-stable, and a combination of heat and pressure is required to eliminate their allergenic potency (Guillamón et al. 2008b).

Genetic improvement

Genetic improvement of nutritive value can modify the content of bioactive compounds. For this reason, it has been possible to genetically eliminate the alkaloids in different species of *Lupinus*, and decrease vicine and convicine in *V. faba* by breeding (von Baer and Vath 1990; Gutierrez et al. 2004).

In classical plant breeding, genetic modifications may occur spontaneously in nature, and humans have consciously tried to exploit these in agriculture for crop improvement and animal husbandry (Duc et al. 1989). Classical plant breeding is more often performed under a controlled environment, making deliberate and specific crosses to maximise the benefits of each parent into a new genotype. This method is only possible where significant genotypic variation exists within the species or within wild-species of the same family.

At present, there are many possibilities for the use of genetically engineered plants. Genetic engineering allows selective modifications by the insertion or elimination of known single genes. Modern genetic manipulation opens up the possibility of acting on the information contained in hereditary material more quickly, adding genes from different species, or modifying gene expression to create genetically modified plants (Monsanto 2001). Over the last few years, transgenic crops have moved from being a laboratory curiosity to providing new varieties grown in large areas throughout the world. Gene transfer protocols and regeneration systems have now been developed for different legume and oilseed species (Babaoglu et al. 2000; Kuchuk et al. 2001). Furthermore, genetic engineering is being used to reduce the content of anti-nutrients such as phytate (Lucca et al. 2001; Raboy 2001), protease inhibitors (Welham and Domoney 2000) and glucosinolates, as well as oestrogen-like compounds, which may lower animal fertility (Greiner and Domoney 2004).

There is no doubt that GMOs and particularly genetically modified plants open an important area that is one of the most promising research areas from the agriculture, food and health perspective. Grain legumes contain seed protein that is relatively deficient in the sulphur amino acids, cysteine and methionine. For instance, in order to increase the concentration of the nutritionally essential sulphur amino acids in seed protein, a transgene encoding a methionine- and cysteine-rich protein, sunflower seed albumin (SSA), was transferred to chickpeas. The results suggested that free methionine and *O*-acetylserine acted as signals that modulated chickpea seed protein composition in response to the variation in sulphur demand, as well as in response to variation in the nitrogen and sulphur status of the plant (Chiaiese et al. 2004). Such transgenic chickpeas have the potential to reduce the amount of synthetic methionine required in chickpea-based diets (Brenes et al. 2004). The nutritive value of transgenic narrow-leafed lupin seed with higher methionine content was also evaluated using broiler chickens (Ravindran et al. 2002).

Nevertheless, considerable protest has been raised regarding the safety of genetically engineered foods. The possibility of allergen transfer by genetic engineering was discovered when a methionine producing gene from Brazil nut (*Bertholletia excelsa*) was incorporated into soybean to increase its protein quality (Greiner and Domoney 2004). The 2S albumin of Brazil nut (allergen Ber e 1) expressed in transgenic soybean is recognized by IgE antibodies of patients who are allergic to Brazil nut (Nordlee et al. 1996). Ewen and Pusztai (1999) investigated the deleterious effects of a GMO potato containing a foreign lectin (*Galanthus nivalis* lectin) on rats. These examples highlight the importance of knowing in advance the characteristics of dietary components (including allergenic properties) whose genes are being transferred to ensure there will be no side-effects. Plant biotechnology is now at the threshold of an exciting new area in which emphasis is on the manipulation of metabolic pathways.

Conclusion

This review makes clear the complexity of these bioactive compounds in legume seeds both from a biochemical and physiological point of view. Taking into account the comparison between beneficial and deleterious effects of these compounds, the most important challenge to be faced in the future is to know their in vivo effects and how to modify their concentrations to our advantage.

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References

Alonso R, Orúe E, Marzo F (1998) Effects of extrusion and conventional processing methods on protein and antinutritional factor contents in pea seeds. Food Chem 63:505–512

Alonso R, Rubio LA, Muzquiz M, Marzo F (2001) The effect of extrusion cooking on mineral bioavailability in pea and kidney bean seed meals. Animal Feed Sci Technol 94:1–13

Alvarez-Alvarez J, Guillamón E, Crespo JF, Cuadrado C, Burbano C, Rodriguez J, Fernandez C, Muzquiz M (2005) Effects of extrusion, boiling, autoclaving and microwave heating on lupin allergenicity. J Agric Food Chem 23:1294–1298

Amstrong WB, Kennedy AR, Wan XS, Atiba J, McLaren E, Meyskens FL (2000) Single-dose administration of Bowman-Birk inhibitor concentrate in patients with oral leukoplakia. Cancer Epidemiol Biom Prev 9:43–47

Aranceta J, Lobo F, Viedma P, Salvador-Castell G, de Victoria EM, Ortega RM, Bello L, Tur-Marí JA (2009) Community nutrition in Spain: advances and drawbacks. Nutr Rev 67(Suppl 1):135–139

Aranda P, Dostalova J, Frias J, Lopez-Jurado M, Kozlowska H, Pokorny J, Urbano G, Vidal-Valverde C, Zdyunczyk Z (2000) Nutrition. In: Hedley CL (ed) Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characteristics. CAB International, Wallingford, pp 61–87

Arora SK (1983) Chemistry and biochemistry of legumes. IFIS Publishing, New Delhi

Asensio-Vegas et al (2007) 6th European conference on grain legumes. Lisbon (Portugal). Abstracts, p 185

Ayet G, Muzquiz M, Burbano C, Robredo LM, Cuadrado C, Price K (1996) Determinación de saponinas en las principales leguminosas cultivadas en España. Food Sci Technol Int 2:96–100

Babaoglu M, Duvey MR, Power JB (2000) Genetic engineering of grain legumes: key transformation events. Ag BiotechNet 2:1–14

Bardocz S, Brown DS, Grant G, Pusztai A, Stewart JC, Palmer RM (1992) Effect of the β-adrenoceptor agonist clenbuterol and phytohemagglutinin on growth, protein-synthesis and polyamine metabolism of tissues of the rat. Br J Pharmacol 106:476–482

Bardocz S, Grant G, Pusztai A, Franklin MF, Carvalho ADFU (1996) The effect of phytohaemagglutinin at different dietary concentrations on the growth, body composition and plasma insulin of the rat. Br J Nutr 76:613–626

Bernabe M, Fenwick R, Frias J, Jimenez-Barbero J, Price K, Valverde S, Vidal-Valverde C (1993) Determination, by NMR spectroscopy, of the structure of ciceritol, a pseudotrisaccharide isolated from lentils. J Agric Food Chem 41(6):870–872

Bohn L, Meyer AS, Rasmussen SK (2008) Phytate: impact on environment and human nutrition. A challenge for molecular breeding. J Zhejiang University Sci B 9:165–191

Brenes A, Jansman AJM, Marquardt RR (2004) Recent progress on research on the effects of antinutritional factors in legume and oil seeds in monogastric animals. In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds. EAAP publication No. 110, Wageningen, pp 195–217

Burbano C, Muzquiz M, Osagie A, Ayet G, Cuadrado C (1995) Determination of phytate and lower inositol phosphates in Spanish legumes by HPLC methodology. Food Chem 62:321–326

Burbano C, Muzquiz M, Ayet G, Cuadrado C, Pedrosa MM (1999) Evaluation of antinutritional factors of selected varieties of *Phaseolus vulgaris*. J Sci Food Agric 79:1468–1472

Campbell PM, Reiner D, Moore AE, Lee RY, Epstein MM, Higgins TJ (2011) Comparison of the α-amylase inhibitor-1 from common bean (*Phaseolus vulgaris*) varieties and transgenic expression in other legumes. Post-translational modifications and immunogenicity. J Agric Food Chem 59(11):6047–6054

Campos-Vega R, Loarca-Piña GF, Oomah BD (2010) Minor components of pulses and their potential impact on human health. Food Res Int 43:461–482

Castonguay Y, Nadeau P, Lechasseur P, Chounard L (1995) Differential accumulation of carbohydrates in alfalfa cultivars of contrasting winterhardiness. Crop Sci 35:509–516

Champ MM (2002) Non-nutrient bioactive substances of pulses. Br J Nutr 88:307–319

Chiaiese P, Ohkama-Ohtsu N, Molving L, Godfree R, Dove D, Hocart C, Fujiwara T, Higgins TJV, Tabe LM (2004) Sulphur and nitrogen nutrition influence the response of chickpea seeds to an added, transgenic sink for organic sulphur. J Exp Bot 55:1889–1901

Clemente A, Mackenzie DA, Johnson IT, Domoney C (2004) Investigation of legume seed protease inhibitors as potential anti-carcinogenic proteins. In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds. EAAP publication No. 110, Wageningen, pp 137–142

Cuadrado C, Ayet G, Robredo LM, Tabera J, Villa R, Pedrosa MM, Burbano C, Muzquiz M (1996) Effect of natural fermentation on the content of inositol phosphates in lentils. Z-Lebensmittel-Undersuchung und-Forschung 203:268–271

Dillard CJ, German JB (2000) Phytochemicals: nutraceuticals and human health. J Sci Food Agric 80:1744–1756

Domoney C (1999) Inhibitors of legume seeds. In: Sewry PR, Casey R (eds) Seed proteins. Kluwer, The Netherlands, pp 697–719

Duc G, Sixdenier G, Lila M, Furstoss V (1989) In: Huisman J, van der Poel AFB, Liener IE (eds) Recent advances of research in antinutritional factors in legume seeds. Wageningen, pp 305–313

Duranti M (2006) Grain legume proteins and nutraceutical properties. Fitoterapia 77:67–82

Egounlety M, Aworth OC (2003) Effect of soaking, dehulling, cooking and fermentation with *Rhizopus oligosporus* on the oligosaccharides, trypsin inhibitor, phytic acid and tannins of soybean (*Glycine max* Merr.), cowpea (*Vigna unguiculata* L. Walp) and groundbean (*Macrotyloma geocarpa* Harms) Jo. J Food Eng 56:249–254

Eiwegger T, Stahl B, Schmitt J, Boehm G, Gerstmayr M, Pichler J, Dehlink E, Loibichler C, Urbanek R, Szépfalusi Z (2004) Human milk-derived oligosaccharides and plant-derived oligosaccharides stimulate cytokine production of cord blood T-cells in vitro. Pediatr Res 56(4):536–540

el-Adawy TA (2002) Nutritional composition and antinutritional factors of chickpeas (*Cicer arietinum* L.) undergoing different cooking methods and germination. Plant Foods Hum Nutr 57:83–97

Elmafda I, Freisling H (2009) Nutritional status in Europe: methods and results. Nutr Rev 61(Suppl. 1):130–134

European Commission Communication (2010) Communication from The Commission to The European Parliament, The Council, The European Economic and Social Committee and the Committee of the Regions. The CAP towards 2020: Meeting the food, natural resources and territorial challenges of the future. http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=COM:2010:0672:FIN:en:PDF. Cited 28 Feb 2012

Ewen SWE, Pusztai A (1999) Effect of diets containing genetically modified potatoes expressing *Galanthus nivalis* lectin on rat small intestine. Lancet 354:1353–1354

Fenwick DE, Oakenfull D (1983) Saponin content of food plants and some prepared foods. J Sci Food Agric 34:186–191

Fenwick GR, Price KR, Tsukamoto C, Okubo K (1991) Saponins. In: D'Mello JPF, Duffus CM, Duffus JH (eds) Toxic substances in crop plants. Royal Society of Chemistry, Cambridge, pp 285–327

Figueroa MOR, Mancini Filho J, Lajolo FM (1984) Ação antinutricional das fito-hemaglutininas de *Phaseolus vulgaris*. Archivos Latinoamericanos de Nutrición 34:489–499

Fleming SE (1981) A study of relationships between flatus potential and carbohydrate distribution in legume seeds. J Food Sci 46:794–798

Gatehouse JA (2011) Prospects for using proteinase inhibitors to protect transgenic plants against attack by herbivorous insects. Curr Protein Pept Sci 12(5):409–416

Gatehouse AMR, Hilder VA, Powell KS, Wang M, Davidson GM, Gatehouse LN, Down RE, Edmons HS, Boulter D, Newell CA, Merryweather A, Hamilton WDO, Gatehouse JA (1994) Insect resistant transgenic plants. Choosing the gene to do the job. Biochem Soc Trans 4:944–949

Goyoaga C, Burbano C, Cuadrado C, Varela A, Guillamón E, Pedrosa MM, Muzquiz M (2008) Content and distribution of vicine, convicine and L-DOPA during germination and seedling growth of two *Vicia faba* L. varieties. Eur Food Res Technol 227:1537–1542

Goyoaga C, Burbano C, Cuadrado C, Varela A, Guillamón E, Pedrosa MM, Muzquiz M (2011) Content and distribution of protein, sugars and phytates during germination and seedling growth of two *Vicia faba* varieties. J Food Compos Anal 24:391–397

Grant G (1991) Legumes. In: D'Mello JPF, Duffus CM, Duffus JH (eds) Toxic substances in crop plants. Royal Society of Chemistry, Cambridge, pp 49–67

Grant G, Murray S, Gravells E, Duguid TJ, Bardocz S, Pusztai A (1998) The body metabolism of mammals can be adversely affected by enzyme inhibitors from legume seeds. In: Jansman AJM, Hill GD, Huisman J, van der Poel AFB (eds) Proceedings of the third international workshop on antinutritional factors in legume seeds and rapeseed. Wageningen, The Netherlands, pp 233–237

Grases F, Costa-Bauza A, Perelló J, Isern B, Vucenik I, Valiente M, Muñoz JA, Prieto RM (2006) Influence of concomitant food intake on the excretion of orally administered myo-inositol hexaphosphate in humans. J Med Food 9:72–76

Greiner R, Domoney C (2004) The potential of genetically modified legume and oilseed crops for food and non-food use. In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds, EAAP publication No. 110, Toledo, pp 261–276

Greiner R, Konietzny U (1996) Phytate hydrolysis in black beans by endogenous and exogenous enzymes In: Bardocz S, Muzquiz M, Pusztai A (eds) Effects of antinutrients on the nutritional value of legume diets. Proceedings of the fourth scientific workshop, Office for Official Publications of the European Communities, Luxembourg, pp 19–26

Greiner R, Konietzny U (2006) Phytase for food application. Food Technol Biotechnol 44:125–140

Greiner R, Pedrosa MM, Muzquiz M, Ayet G, Cuadrado C, Burbano C (1998) Effect of germination on phytate content and phytase activity in legumes. In: 3rd European conference on grain legumes, Valladolid, pp 82–83

Greiner R, Larsson-Alminger M, Carlsson N-G, Muzquiz M, Burbano C, Cuadrado C, Pedrosa MM, Goyoaga C (2002) Pathway of dephosphorylation of myo-inositol hexakisphosphate by phytases of legume seeds. J Agric Food Chem 50:6865–6870

Guillamón E, Pedrosa MM, Burbano C, Cuadrado C, Sanchez M, Muzquiz M (2008a) The trypsin inhibitors present in seed of different grain legume species and cultivar. Food Chem 107:68–74

Guillamón E, Burbano C, Cuadrado C, Muzquiz M, Pedrosa MM, Sanchez M (2008b) Effect of instant controlled pressure drop on in vitro lupin allergenicity to lupins (*Lupinus albus* var. Multolupa). Int Archives Allergy Immunol 145:9–14

Gutierrez N, Duc G, Marget P, Avila CM, Suso MJ, Cubero JI, Moreno MT, Torres AM (2004) Identification of molecular markers tightly linked to low tannin and vicine-convicine content in faba beans. In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds. EAAP publication No. 110, Wageningen, pp 287–290

Haddad J, Allaf K (2007) A study of the impact of instantaneous controlled pressure drop on the trypsin inhibitors of soybean. J Food Eng 79:353–357

Haddad J, Muzquiz M, Allaf K (2006) Treatment of lupin seed using the instantaneous controlled pressure drop technology to reduce alkaloid content. Food Sci Technol Int 12:365–370

Haddad J, Greiner R, Allaf K (2007) Effect of instantaneous controlled pressure drop on the phytate content of lupin. Swiss Soc Food Sci Technol 40:448–453

Hajós G, Osagie AU (2004) Technical and biotechnological modifications of antinutritional factors in legume and oilseeds In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds, EAAP publication No. 110, Toledo, pp 293–305

Hajós G, Gelencsér E, Pusztai A, Bardocz S (1996) Measurements of the antinutrient contents of legume samples. In: Bardocz S, Gelencser E, Pusztai A (eds) COST98-effects of antinutrients on the nutritional value of legume diets, vol I. Office for Official Publications of the European Communities, Luxembourg, pp 130–134

Haros M, Bielecka M, Honke J, Sanz Y (2007) Myo-inositol hexakisphosphate degradation by *Bifidobacterium infantis* ATCC 15697. Int J Food Microbiol 117:76–84

Hill GD (2004) Grain legumes and oilseeds-the way ahead. In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds, EAAP publication No. 110, Toledo, pp 353–364

Hill JE, Kysela D, Elimelech M (2007) Isolation and assessment of phytate-hydrolysing bacteria from the DelMarVa Peninsula. Environ Microbiol 9:3100–3107

Jaffe WG, Moreneo R, Wallis V (1973) Amylase inhibitors in legume seeds. Nutr Rep Int 7:169–174

Jenab M, Thompson LU (2002) Role of phytic acid in cancer and other diseases. In: Reddy NR, Sathe SK (eds) Food phytates. CRC Press, Boca Raton, pp 225–248

Jiménez-Martinez C, Pedrosa MM, Muzquiz M, Dávila-Ortiz G (2004) Elimination of quinolizidine alkaloids, α-galactosides and phenolic compounds from *Lupinus campestris* seed by aqueous, acid and alkaline thermal treatment In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds, EAAP publication No. 110, Toledo, pp 343–346

Kadlec P, Bjergegaard C, Gulewicz K, Horbowicz M, Jones A, Kintia P, Kratchanov C, Kratchanova M, Lewandowicz G, Soral-Smietana M, Sorensen H, Urban J (2000) Carbohydrate chemistry. In: Hedley CL (ed) Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characteristics. CAB International, Wallingford, pp 15–59

Kennedy AR (1995) The evidence for soybean products as cancer preventive agents. J Nutr 125:733S–743S

Kennedy AR, Wan XS (2002) Effects of the Bowman-Birk inhibitor on growth, invasion, and clonogenic survival of human prostate epithelial cells and prostate cancer cells. Prostate 50:125–133

Kennedy AR, Billings PC, Wan XS, Newberne PM (2002) Effects of Bowman-Birk inhibitor on rat colon carcinogenesis. Nutr Cancer 43:174–186

Konietzny U, Greiner R (2003) Phytic acid: nutritional impact. In: Caballero B, Trugo L, Finglas P (eds) Encyclopaedia of food science and nutrition. Elsevier, London, pp 4555–4563

Koratkar R, Rao AV (1997) Effect of soya bean saponins on azoxymethane-induced preneoplastic lesions in the colon of mice. Nutr Cancer 27:206–209

Kozlowska H, Aranda P, Dostalova J, Frias J, Lopez-Jurado M, Kozlowska H, Pokorny J, Urbano G, Vidal-Valverde C, Zdyunczyk Z (2001) Nutrition. In: Hedley CL (ed) Carbohydrates in grain legume seeds: improving nutritional quality and agronomic characters. CABI Publishing, Oxon, p 352

Kuchuk N, Griga M, Kusturkova G, Ilieva-Stoilova M (2001) Biotechnology. In: Hedley CL (ed) Carbohydrates in grain legume seeds. CAB International, Wallingford, pp 145–226

Kumar V, Sinha AK, Makkar HPS, Becker K (2010) Dietary roles of phytate and phytase in human nutrition: a review. Food Chem 120:945–959

Kuriyama S, Mendel LB (1917) The physiological behaviour of raffinose. J Biol Chem 31:125–147

Lajolo FM, Genovese MI (2002) Nutritional significance of lectins and enzyme inhibitors from legumes. J Agric Food Chem 50:6592–6598

Lajolo FM, Genovese MI, Pryme IF, Dale M, (2004) Beneficial (antiproliferative) effects of different substances. In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds, EAAP publication No. 110, Wageningen, pp 123–135

Lasheras C, Fernandez S, Patterson AM (2000) Mediterranean diet and age with respect to overall survival in institutionalized, non-smoking elderly people. Am J Clin Nutr 71:987–992

Le Berre-Anton V, Bompard-Gilles C, Paya F, Rouge P (1997) Characterization and functional properties of the α-amylase inhibitor (α-AI) from kidney bean (*Phaseolus vulgaris*) seeds. Biochim Biophys Acta 1343:31–40

Leterme P (2002) Recommendations by health organizations for pulse consumption. Br J Nutr 88:5239–5242

Loewus F (2002) Biosynthesis of phytate in food grains and seeds. In: Reddy NR, Sathe SK (eds) Food phytates. CRC Press, Boca Raton, pp 53–61

Lopez HW, Leenhardt F, Coudray C, Rémésy C (2002) Minerals and phytic acid interactions: is it a real problem for human nutrition? Int J Food Sci Technol 37:727–739

Lucca P, Hurrell R, Potrycus I (2001) Genetic engineering approaches to improve the bioavailability and the level of iron in rice grains. Theor Appl Genet 102:392–397

Mahungu SM, Diaz-Mercado S, Li J, Schwenk M, Singletary K, Faller J (1999) Stability of isoflavones during extrusion processing of corn/soy mixture. J Agric Food Chem 47:279–284

Maiti IB, Majumber AL, Biswas BB (1974) Purification and mode of action of phytase from *Phaseolus aureus*. Phytochemistry 13:1047–1051

Martín-Cabrejas MA, Sanfiz B, Vidal A, Mollá E, Esteban R, López-Andréu FJ (2004) Effect of fermentation and autoclaving on dietary fiber fractions and antinutritional factors of beans (*Phaseolus vulgaris* L.). J Agric Food Chem 52:261–266

Martínez-Villaluenga C, Frias J, Vidal-Valverde C (2008) Alpha-galactosides: antinutritional factors or functional ingredients? Crit Rev Food Sci Nutr 48:301–316

Marzo F, Alonso R, Urdaneta E, Arricibita FJ, Ibañez F (2001) Nutritional quality of extruded kidney bean (*Phaseolus vulgaris* L. var. Pinto) and its effects on growth and skeletal muscle nitrogen fractions in rats. J Anim Sci 80:875–879

Minihane AM, Rimbach G (2002) Iron absorption and the iron binding and anti-oxidant properties of phytic acid. Int J Food Sci Technol 37:741–748

Mitsuoka T (1996) Intestinal flora and human health. Asia Pac J Clin Nutr 5:2–9

Monsanto (2001) In: Cuaderno Técnico n°1. Monsanto Agricultura España, S.L. (ed)

Mulimani VH, Rudrappa G, Supriya D (1994) α-Amylase inhibitors in chick pea (*Cicer arietinum* L). J Sci Food Agric 64:413–415

Muzquiz M (2000) Factores antinutricionales en fuentes proteicas. In: Vioque J, Clemente A, Bautista J, Millán F (eds) Jornada internacional sobre proteínas alimentarías. Sevilla, Spain

Muzquiz M, Wood JA (2007) Antinutritional Factors. In: Yadav SS, Redden R, Chen W, Sharma B (eds) Chickpea breeding & management. CABI, Wallingford, pp 143–166

Muzquiz M, Cuadrado C, Ayet G, Robredo LM, Pedrosa MM, Burbano C (1996) Changes in non-nutrient compounds during germination. In: Bardocz S, Gelencser, E, Pusztai A (eds) Effects of antinutrients on the nutritional value of legume diets. Proceedings of the second scientific workshop in Budapest, Budapest, pp 124–129

Muzquiz M, Burbano C, Ayet G, Pedrosa MM, Cuadrado C (1999a) The investigation of antinutritional factors in *Phaseolus vulgaris*. Environmental and varietal differences. Biotechnol Agron Soc Environ 3(4):210–216

Muzquiz M, Burbano C, Pedrosa MM, Ciesiołka D, Pilarski R, Gulewicz K (1999b) Studies on the influence of different nitrogen forms on the chemical composition of various cultivars of *Lupinus albus* L. Commun Soil Sci Plant Anal 40:2009–2027

Muzquiz M, Burbano C, Cuadrado C, Martin M (2001) Analytical methods for determination of compounds with no nutritive value. In: Jacobsen HJ, Muzquiz M, Hassa A (eds) Handbook on common bean related laboratory methods. Galicia, Spain, pp 11–26

Muzquiz M, Cuadrado C, Guillamón E, Goyoaga C, Altares P, Varela A, Pedrosa MM, Burbano C (2003) Implicación en nutrición y salud de compuestos tóxicos y no-nutritivos de leguminosas. In: de Andalucía Junta (ed) 1as Jornadas de la Asociación Española de Leguminosas. Córdoba, Spain, pp 80–82

Muzquiz M, Burbano C, Pedrosa MM, Ciesiołka D, Pilarski R, Gulewicz K (2009) Studies on the influence of different nitrogen forms on the chemical composition of various cultivars of *Lupinus albus* L. Com Soil Sci Plant Anal 40:2009–2027

Muzquiz M, Guillamón E, Burbano C, Pascual H, Cabellos B, Cuadrado C, Pedrosa MM (2011) Chemical composition of a new *Lupinus* species found in Spain, *Lupinus mariae-josephi* H. Pascual (Fabaceae). Span J Agric Res 9(4): 1233–1244

Naveeda K, Jamuna P (2004) Nutritional quality of microwave-cooked and pressure-cooked legumes. Int J Food Sci Nutr 55:441–448

Nishida C, Uauy R, Kumanyika S, Shetty P (2004) The Joint WHO/FAO Expert Consultation on diet, nutrition and the prevention of chronic diseases: process, product and policy implications. Public Health Nutrition 7:245–250

Nishino H, Murakoshi M, Masuda M, Tokuda H, Satomi Y, Onozuka M, Yamaguchi S, Bu P, Tsuruta A, Nosaka K, Baba M, Takasuka N (1999) Suppression of lung and liver carcinogenesis in mice by oral administration of myo-inositol. Anticancer Res 19:3663–3664

Nordlee JA, Taylor SL, Townsend JA, Thomas LA, Bush RK (1996) Identification of a Brazil-nut allergen in transgenic soybeans. N Engl J Med 14:688–692

Oakenfull DG, Sidhu G (1989) Saponins. In: Cheeke PR (ed) Toxicants of Plant Origin, vol II. Glycosides. CRC Press Inc, Boca Raton, p 131

Pedrosa MM, Cuadrado C, Burbano C, Allaf K, Haddad J, Gelencsér E, Takács K, Guillamón E, Muzquiz M (2012) Effect of instant controlled pressure drop on the oligosaccharides, inositol phosphates, trypsin inhibitors and lectins contents of different legumes. Food Chem 131:862–868. doi:10.1016/j.foodchem.2011.09.061

Price KR, Johnson IT, Fenwick RG (1987) The chemistry and biological significance of saponins in foods and feedingstuff. CRC Critical Reviews in Food Science and Nutrition 26:27–131

Price KR, Eagles J, Fenwick GR (1988) Saponin composition of 13 varieties of legume seed using fast atom bombardment mass-spectrometry. J Sci Food Agric 42:183–193

Pryme IF, Pustzai A, Bardocz S, Ewen SWB (1998) The induction of gut hyperplasia by phytohaemagglutinin in the diet and limitation of tumour growth. Histol Histopathol 13:575–583

Pusztai A, Grant G, Duguid TJ, Brown DS, Peumans WJ, van Damme EJM, Bardocz S (1995) Inhibition of starch digestion by an α-amylase inhibitor reduces the efficiency of utilisation of dietary proteins and lipids and retards the growth of rats. J Nutr 125:1554–1562

Pusztai A, Grant G, Buchan WC, Bardocz S, de Carvalho ADFU, Ewen SWB (1998) Lipid accumulation in obese Zucker rats is reduced by inclusion of raw kidney bean (*Phaseolus vulgaris*) in the diet. Br J Nutr 79:213–221

Pusztai A, Bardocz S, Martín-Cabrejas M (2004) The mode of action of ANFs on the gastrointestinal tract and its microflora. In: Muzquiz M, Hill GD, Pedrosa MM, Burbano C (eds) Proceedings of the fourth international workshop on antinutritional factors in legume seeds and oilseeds. EAAP publication No. 110, Wageningen, pp 87–100

Quemener B, Brillouet JM (1983) Ciceritol, a pinitol digalactoside from seeds of chickpea, lentil and white lupin. Phytochemistry 22:1745–1751

Raboy V (2001) Seeds for a better future:”low phytate” grains help to overcome malnutrition and reduce pollution. Trends Plant Sci 6:458–462

Rajkó R, Szabó G, Vidal-Valverde C, Kovács E (1997) Designed experiments for reducing antinutritive agents in soybean by microwave energy. J Agric Food Chem 45:3565–3569

Rao PU, Belavady B (1978) Oligosaccharide in pulses: Varietal differences and effects of cooking and germination. J Agric Food Chem 26:316–319

Rao PU, Deosthale YG (1982) Tannin contents of pulses: Varietal differences and effect of cooking and germination. J Sci Food Agric 33:1013–1016

Ravindran V, Tabe LM, Molvig L, Higgins TVJ, Bryden WL (2002) Nutritional evaluation of transgenic high-methionine lupins (*Lupinus angustifolius* L) with broiler chickens. J Sci Food Agric 82:280–285

Rehman ZU, Shah WH (1998) Effect of microwave and conventional cooking on some nutrients, antinutrient and protein digestibility of chick-peas (*Cicer arietinum*). Bangladesh Journal of Scientific and Industrial Research 33:200–205

Rehman ZU, Shah WH (2005) Thermal heat processing effects on antinutrients, protein and starch digestibility of food legumes. Food Chem 91:327–331

Reyes-Moreno C, Cuevas-Rodriguez EO, Milan-Carrillo J, Cardenas-Valenzuela OG, Barron-Hoyos J (2004) Solid state fermentation process for producing chickpea (*Cicer arietinum* L.) tempeh flour. Physicochemical and nutritional characteristics of the product. J Sci Food Agric 84:271–278

Rimbach G, Pallauf J (1997) Cadmium accumulation, zinc status, and mineral bioavailability of growing rats fed diets high in zinc with increasing amounts of phytic acid. Biol Trace Elem Res 57:59–70

Rimbach G, Pallauf J, Walz OP (1996) Effect of microbial phytase on cadmium accumulation in pigs. Archives fur Tierernahrung 49:279–286

Roberts MF, Wink M (1998) Introduction. In: Roberts MF, Wink M (eds) Alkaloids. biochemistry, ecology, and medicinal applications. Plenum Press, New York, pp 1–7

Rochfort S, Panozzo J (2007) Phytochemicals for health, the role of pulses. J Agric Food Chem 55:7981–7994

Rubio LA, Pedrosa MM, Cuadrado C, Gelencser E, Clemente A, Burbano C, and Muzquiz, M (2006) Recovery at the terminal ileum of some legume non-nutritional factors in cannulated pigs. J Sci Food Agric 86(6):979–987

Ruiz RG, Price KR, Arthur AE, Rose ME, Rhodes MJ, Fenwick RG (1996a) Effect of soaking and cooking on the saponin content and composition of chickpeas (*Cicer arietinum*) and lentils (*Lens culinaris*). J Agric Food Chem 44:1526–1530

Ruiz RG, Price K, Rose M, Rhodes M, Fenwick R (1996b) A preliminary study on the effect of germination on saponin content and composition of lentils and chick peas. Zeitschrift fur Lebensmittel- Untersuchung und-Forschung 203(4):366–369

Ruiz RG, Price KR, Fenwick RG, Rhodes MJC (1997) Changes in saponin content and composition of chickpeas and lentils during germination and cooking. In: Bardocz S, Muzquiz M, Pusztai A (eds) Effects of antinutrients on the nutritional value of legume diets. Office for Official Publications of the European Communities, Luxembourg, Proceedings of the Fourth Scientific Workshop, pp 15–18

Rupérez P (1998) Oligosaccharides in raw and processed legumes. Zeitschrift für Lebensmitteluntersuchung und Forschung A 206(2):130–136

Sánchez MC, Altares P, Pedrosa MM, Burbano C, Cuadrado C, Goyoaga C, Muzquiz M, Jimenez-Martinez C, Dávila-Ortiz G (2005) Alkaloid variation during germination in different lupin species. Food Chem 90:347–355

Sandberg AS (2002) Bioavailability of minerals in legumes. Br J Nutr 88(Suppl 3):281–285

Sandberg AS, Andersson H, Carlsson NG, Sandström B (1993) Degradation of soy bean oligosaccharides in the stomach and small intestine of human ileostomy subjects. In: Schlemmer U (ed) Proceedings of the International Conference on Bioavailability 93- Nutritional. Chemical and Food Processing Implications of Nutrient Availability, Karlsruhe, pp 197–201

Saxena AK, Chadha M, Sharma S (2003) Nutrients and antinutrients in chickpea (*Cicer arietinum* L.) cultivars after soaking and pressure cooking. J Food Sci Technol 40:493–497

Schley PD, Field CJ (2002) The immune-enhancing effects of dietary fibres and prebiotics. Br J Nutr 87(Suppl 2):221–230

Shi J, Arunasalam K, Yeung D, Kakuda Y, Mittal G, Jiang Y (2004) Saponins from edible legumes: chemistry, processing, and health benefits. J Med Food 7:67–78

Shibuya M, Hoshino M, Katsube Y, Hayashi H, Kushiro T, Ebizuka Y (2006) Identification of β-amyrin and sophoradiol 24-hydroxylase by expressed sequence tag mining and functional expression assay. FEBS J 273:948–959

Singh U, Kherdekar MS, Jambunathan R (1982) Studies on desi and kabuli Chickpea (*Cicer arietinum* L.) cultivars. The levels of amylase inhibitors, levels of oligosaccharides and in vitro starch digestibility. J Food Sci 47:510–512

Sofi F, Cesari F, Abbate R, Gensini GF, Casani A (2008) Adherence to Mediterranean diet and health status: meta-analysis. Br Med J 337:a1344

Srinivasan A, Giri AP, Harsulkar AM, Gatehouse JA, Gupta VS (2005) A Kunitz trypsin inhibitor from chickpea (*Cicer arietinum* L.) that exerts anti-metabolic effect on podborer (*Helicoverpa armigera*) larvae. Plant Mol Biol 57:359–374

Steer TE, Gibson GR (2002) The microbiology of phytic acid metabolism by gut bacteria and relevance for bowel cancer. Int J Food Sci Technol 37:783–790

Tava A, Oleszek W, Jurzysta M, Berardo N, Odoardi M (1993) Alfalfa saponins and sapogenins: isolation and quantification in two different cultivars. Phytochem Anal 4:269–274

Thompson LU (1993) Potential health benefits and problems associated with antinutrients in foods. Food Res Int 26:131–149

Trugo LC, Muzquiz M, Pedrosa MM, Ayet G, Burbano C, Cuadrado C, Cavieres E (1999) Influence of malting on selected components of soya bean, black bean, chickpea and barley. Food Chem 65(1):85–90

UNESCO (2010) Fifth Session of the UNESCO's Intergovernmental committee for the safeguarding of the intangible cultural heritage. Decision 5.COM 6.41 Nairobi, Kenya, 15–19 November 2010 http://www.unesco.org/culture/ich/doc/src/ITH-10-5.COM-CONF.202-Decisions-EN.doc. Cited 28 Feb 2012

Urbano G, López-Jurado M, Aranda P, Vidal-Valverde C, Tenorio EJ, Porres E (2000) The role of phytic acid in legumes: antinutrient or beneficial function? Journal of Physiology and Biochemistry 56:283–294

Urdaneta E, Alberdi J, Aranguren P, Barrenetxe J, Pedrosa MM, Marzo F (2003) Efecto del extrusionado de Lupinus (*Lupinus albus* L. var. Multolupa) en ratas en crecimiento. In: de Andalucía Junta (ed) 1as Jornadas de la Asociación Española de Leguminosas. Spain, Córdoba, pp 115–116

von Baer E, Vath D (1990) New varieties of Lupin. In: von Baer E (ed) Proceedings of the 6th international Lupin conference. International Lupin Association (ILA), Temuco-Pucon, pp 376–381

Wattenberg LW, Wiedmann TS, Estensen RD, Zimmerman CL, Galbraith AR, Steele VE, Kelloff GJ (2000) Chemoprevention of pulmonary carcinogenesis by brief exposures to aerosolized budesonide or beclomethasone dipropionate and by the combination of aerosolized budesonide and dietary myo-inositol. Carcinogenesis 21:179–182

Welham T, Domoney C (2000) Temporal and spatial activity of a promoter from a pea enzyme inhibitor gene and its exploitation for seed quality improvement. Plant Sci 159:289–299

Whitaker JR (1988) α-Amylase inhibitors of higher plants and microorganisms. In: Food proteins. Kinsella JE, Soucle WG (eds) Proceedings of the protein. Co-products Symposium, American Oil Chemists' Society, Champaign II, USA, pp 354–380

WHO (2003) Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series No. 916 World Health Organization: Geneva

Wiggins HS (1984) Nutritional value of sugars and related compounds undigested in the small intestine. Proceedings of the Nutrition Society 43:69–75

Woldemichael GM, Montenegro G, Timmermann BN (2003) Triterpenoidal lupin saponins from the Chilean legume Lupinus oreophilus Phil. Phytochemistry 63:853–857

Wood JA, Harden S (2006) A method to estimate the hydration and swelling properties of chickpeas (*Cicer arietinum* L.). J Food Sci 71:E190–E195

Zdunczyk Z, Juskiewicz J, Frejnagel S, Gulewicz K (1998) Influence of alkaloid and oligosaccharides from white lupin seeds on utilization of diets by rats and absorption of nutrients in the small intestine. J Anim Feed Sci 72:143–154

Zhou JR, Erdman JW (1995) Phytic acid in health and disease. Crit Rev Food Sci Nutr 35:495–508

Zucoloto S, Scaramello AC, Lajolo FM, Muccillo G (1991) Effect of oral phytohemagglutinin intake on cell adaptation in the epithelium of the small intestine of the rat. Int J Exp Pathol 72:41–45