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# Non-nutrient bioactive substances of pulses

Martine M.-J. Champ*

*National Institute for Agronomic Research (INRA), Human Nutrition Research Centre, Rue de la Géraudière, BP 71627, 44316 Nantes Cedex 03, France*

Pulses supply many bioactive substances found in minor amounts in food, but which may have significant metabolic and/or physiological effects. These compounds have long been classified as antinutritional factors, but many studies have reconsidered their impact on health. Some could play a role in the prevention of the major diseases of affluent societies. As these compounds can be beneficial or adverse, depending on conditions, an assessment of their various physiological effects is necessary to determine whether they should be preserved or eliminated in each main nutritional situation.

**Pulses: Bioactive substances: Antinutritional factors: Humans**

## Introduction

Pulses contain a number of bioactive substances (Table 1) that cannot be considered as nutrients, but which exert metabolic effects on the humans or animals that consume these food forms. These effects, which are generally observed when pulses are consumed on a regular basis, may be regarded as positive, negative or both. Most bioactive substances have been classified as ‘antinutritional factors’ and are referred to by many different terms in the literature: toxic constituents, toxins or food toxicants (Liener, 1976, 1980; Rechcigl, 1983), antinutrients or antinutritional factors (or compounds) (Thompson, 1993; van der Poel *et al.* 1993; Gatel & Champ, 1998; Gaudard-de Weck, 1998; Frøkiær *et al.* 2001), bioactive substances, nutritive factors (Savage & Deo, 1989), associated substances, micronutrients and phytochemicals (Ferguson & Harris, 1999). The terms ‘bioactive substances or compounds’ will be used in the present paper.

The purpose of the present paper is to describe these compounds, indicate their beneficial and adverse effects, and determine their concentration in the main European pulses before and after classical cooking procedures (their activity often depends on the treatments applied to pulses before ingestion). The discussion will assess the positive and negative properties of each of these compounds.

## Main non-nutrient bioactive compounds of pulses

### *Enzyme inhibitors*

Among the enzyme inhibitors of pulses, trypsin and chymotrypsin inhibitors elicited the largest number of studies in the 1970s and 1980s, due to their impact on animal nutrition. Both of these groups of inhibitors are proteins. They are abundant in raw cereals and legumes (Table 2), but can be inactivated by hydrothermic treatments. Trypsin inhibitors might deplete the sulphur-containing amino acids that are already scarce in most grain legumes.

Trypsin inhibitor activity (TIA) is quite different from one cultivar to another. An extensive study of 195 pea cultivars showed a variation in seed TIA of 1 to 15 units/mg dry matter (Page *et al.* 2001). French winter pea cultivars and some British cultivars (Maro, Progreta) have high TIA levels (Leterme *et al.* 1992). According to Castaing & Leuillet (1981), winter varieties have three times the content of trypsin inhibitors found in spring varieties. It is quite difficult to compare the data available in the literature, as the methods and units used often differ (Table 2).

Protease inhibitors are known to defend the plant against predators. Consequently, removal of these products by breeding could have deleterious effects for the plant.

α-Amylase inhibitors were first reported in buckwheat and later in many other grains, tubers, fruits and legumes (Table 2). They are also proteins, sensitive to hydrothermic treatments. With few exceptions, most amylase inhibitors from plants are active against animal amylases, but inactive against bacterial, fungal and plant enzymes. The inhibitor forms a complex with the amylase, the extent of which depends on a number of factors, including pH, ionic strength, temperature, time of interaction and inhibitor concentration. The complex formation can inactivate amylase and, in turn, cause a reduction in starch digestion (Thompson, 1993).

**Abbreviations:** ACF, aberrant crypt foci; $IP_6$, phytic acid; TIA, trypsin inhibitor activity.

* **Corresponding author:** Dr Martine M.-J. Champ, fax +33 (0)2 40 67 50 12, email champ@nantes.inra.fr

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**Table 1.** Main non-nutrient bioactive pulse compounds and their main potential positive and beneficial effects

| | Beneficial effects | Adverse effects | Amount in untreated pulses* | Main source(s) |
|---|---|---|---|---|
| Protease inhibitors | Anticarcinogenic (?) | ↑ Carcinogenesis (?) and growth inhibition (in animals) | +++ | Soya, GL, cereals |
| Amylase inhibitors | Potentially therapeutic in diabetes (?) | ↓ Starch digestion | +++ | Cereals, GL |
| Lectins | May help in obesity treatment (??), ↓ tumour growth (??) | Growth inhibition (in animals), ↓ nutrient absorption | ++(+) | Beans |
| Phytates | Hypocholesterolaemic effect (?), anticarcinogenic (?) | ↓ Bioavailability of minerals | ++ | Wheat bran, soya, GL |
| Oxalates | | ↓ Bioavailability of minerals | + | Spinach, rhubarb, beans |
| Phenolic compounds | | | | |
| Flavonoids, isoflavones (phyto-oestrogens) | ↓ Risk factors for menopause (CHD..) (?), ↓ risk of hormone-dependent cancer (?) | Infertility syndrome (in animals) | + | Soya, clover |
| Condensed tannins | | Astringent taste, ↓ food intake (in animals) | ++ | Tea, sorghum, rapeseed, *Vicia faba* |
| Lignans (phyto-oestrogens) | ↓ Risk factors for menopause (?) | | | Linseed |
| Lignins | | ↓ Fermentability of dietary fibres | + | Straw |
| Saponins | Hypocholesterolaemic effect (?), anticarcinogenic (?) | Bitter taste, ↓ food intake (in animals) | ++(+) | Lucerne (alfalfa), ginseng |
| Alkaloids | | | | Lupin |

CHD, coronary heart disease; GL, grain legumes.
* Compared to main sources.

### *Lectins or phytohaemagglutinins*

Lectins or haemagglutinins (or phytohaemagglutinins) are found in most plant foods, including those that may be eaten without heat treatment or processing, such as common salad ingredients or fresh fruits (Nachbar & Oppenheim, 1980). However, grain legumes are the main sources of lectins in ordinary human food. Beans (most species, including *Phaseolus vulgaris*) seem to be important sources of lectins, but some varieties can have a much higher lectin content than others (Bond & Duc, 1993). As a result, residual quantities of the initial levels may resist even normal cooking at altitudes well above sea level (de Muelenaere, 1965).

Lectins are sugar-binding proteins that are able to bind and agglutinate red blood cells. They are specific not only in the sugars that they bind to on cell membranes, but also in their toxicity.

The same methodological problems noted with enzyme inhibitors make it difficult to compare data in the literature (Table 2).

### *Phytates and oxalates*

Legumes serve as a dietary source of minerals, although their bioavailability is considered to be lower than that of other foods because of phytates and oxalates (Sandberg, 2002). Phytic acid (myo-inositol 1,2,3,4,5,6, hexakis-dihydrogen phosphate; $IP_6$) is the major storage form of phosphorus in plants (60–90 % of total seed phosphorus). It is a ubiquitous seed constituent, comprising 1–3 % of all nuts, cereals, legumes and oil seeds (Graf, 1986). The amount found in common pulses is apparently lower than 2 % (Table 2). It is present as globoid crystals inside protein bodies located in discrete regions of seeds, such as the aleurone layer of wheat and rice (Graf, 1986). Phytic acid has high ability to chelate multivalent metal ions, especially Zn, Ca and Fe.

During food processing and digestion, phytates can be dephosphorylated to produce degradation products such as myo-inositol pentakis ($IP_5$), tetrakis ($IP_4$), tris- ($IP_3$), bis- ($IP_2$) and monophosphates ($IP_1$).

Oxalic acid [$(COOH)_2$] is present in many plants and vegetables, notably those of the *Oxalis* and *Rumex* families, in which it occurs in the cell sap of the plant in the form of K or Ca salts. It is also found in small amounts in most pulses (Table 2). Like phytates, oxalates reduce mineral bioavailability and are therefore considered to be antinutritional compounds (Hazell & Johnson, 1987; Proulx *et al.* 1993).

### *Phenolic compounds*

Polyphenolic compounds are widely distributed in the plant world and are involved in protection mechanisms against various bacterial, fungal, viral or chemical attacks. The phenolic acids are either derivatives of benzoic acid (e.g.

**Table 2.** Amount of non-nutrient bioactive substances in the main pulse species compared to a single main source (% dry matter basis except when otherwise indicated)

| | *Phaseolus vulgaris* | *Lens esculenta* | *Cicer arietinum* | *Pisum sativum* | *Vicia faba* | *Lupinus albus* | *Glycine max* (or other reference when indicated) |
|---|---|---|---|---|---|---|---|
| Trypsin inhibitor activity* | | | | | | | |
| TIU units/mg DM | 9·6 | 8·4 | 1–15 | 5·4–7·8 | 6·7 | <1 | |
| TIA/g | 0·425 | 0·178 | | | | | 0·415 |
| mg/g | | | | 4·4–12·5 | | | |
| U/g DM | | | | 2700–11700 | | | |
| Chymotrypsin inhibitor activity (IU/g)† | | | | 740–10240 | 380–770 | | |
| Amylase inhibitor activity (U/g)‡ | | | | 14–80 | | | |
| Haemagglutinin activity§ | | | | | | | |
| HA | 8200 | 640 | 0 | 80 | | | |
| HU/mg | | | | 100–400 | 25–100 | | |
| U/g | 2450–3560 | | | 5100–15060 | | | |
| µg | | | | 2·5–5·0 | 10–20 | 10 000 | 0·3–1·2 |
| Phytates‖ | 0·2–1·9 | 0·4–0·7 | 0·4–1·1 | 0·2–1·3 | 0·5–1·1 | | 2·5–5·5 (wheat bran) |
| Oxalates¶ | 0·10–0·5 | 0·16 | 0·07 | (0·7) | | | 9·8 (spinach) |
| Polyphenols** | | | | | | | |
| Total | 0·0–0·4 | 1·0 | 0·1–0·6 | 0·25 | 1·1 | | >10 (sorghum) |
| Phenolic acid | | | | 0·001–0·003 | | | |
| Tannins | 0·0–0·7 | 0·1 | 0·0–0·1 | 0·0–1·3 | 0·0–2·1 | | |
| Isoflavones (mg/100 g) | 1–7 | | | | | | 130–180 |
| Daidzein (mg/100 g) | 0·01–0·04 | 0·00–0·01 | 0·01–0·19 | 0·00–0·05 | | | 11–85 |
| Genistein (mg/100 g) | 0·01–0·52 | 0·01–0·02 | 0·07–0·21 | 0·00–0·05 | | | 20–103 |
| Lignans (mg/100 g) | 0·3 | 1·8 | | | | | 0·9 |
| Secoisolariciresinol (mg/100 g) | 0·06–0·15 | 0–0·01 | 0·01 | 0·00–0·01 | | | 0·01–0·27 |
| Saponins†† | | 0·4–0·5 | 0·4 | 0·1–0·3 | 0·4 | | 0·6 |

DM, dry matter; TIU, trypsin inhibitor units; TIA, trypsin inhibitor activity; U, Units, IU, International Units; HA, haemagglutinin activity; HU, haemagglutinating units.

* Liener (1976), Melcion &Valdebouze (1977), Viroben (1979), Gueguen *et al.* (1980), Valdebouze *et al.* (1980), Ekpenyoung & Borchers (1981), Bertrand *et al.* (1982), Lacassagne *et al.* (1988), Huisman (1990), Jondreville *et al.* (1992), Zdunczyk *et al.* (1997), Chrenkova *et al.* (2001), Page *et al.* (2001), Smulikowska *et al.* (2001).

† Savage & Deo (1989).

‡ Savage & Deo (1989).

§ Melcion & Valdebouze (1977), Liener (1979), Viroben (1979), Gueguen *et al.* (1980), Valdebouze *et al.* (1980), Ekpenyoung & Borchers (1981), Bertrand *et al.* (1982), Savage & Deo (1989).

‖ Besançon (1978), Ellis & Morris (1982), Maga (1982), Savage & Deo (1989), Proulx *et al.* (1993), Quinteros *et al* (1999), Carbonaro *et al.* (2001), Vidal-Valverde *et al.* (2001).

¶ Savage & Deo (1989) Quinteros *et al* (1999).

** Savage & Deo (1989), Longstaff & McNab (1991), Saini (1993), Zdunczky *et al.* (1997), Mazur (1998), Bingham *et al* (1998), Bravo (1998), Horn-Ross *et al.* (2000), Liggins *et al.* (2000), Carbonaro *et al.* (2001), Smulikowska *et al.* (2001).

†† Savage & Deo (1989).

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gallic, syringic and vanillic acid) or of cinnamic acid (e.g. caffeic, ferulic, sinapic and *p*-coumaric acid), which are commonly found as esters of caffeic and quinic acids.

Among polyphenolic compounds, flavonoids (e.g. catechin, epicatechin, etc.) are the most common and widely distributed group of plant phenolics. They are the monomeric constituents of condensed tannins, but are also very common as free monomers. They comprise more than 3000 compounds, including anthocyans (255), isoflavonoides (630), or flavones and flavonols (1660). Their common structure is that of diphenylpropanes (C6–C3–C6), consisting of two aromatic rings linked by three carbons that usually form an oxygenated heterocycle. Flavonoids occasionally occur as aglycones, although they are most commonly found as glycoside derivatives. Flavonol is derived from catechin, the basis of condensed tannins in wine. According to some authors, they are involved in the so-called ‘French paradox’.

Among flavonoids, isoflavones (e.g. genistein or daidzein), in which ring B of the flavone molecule is attached to carbon 3 of the heterocycle, occur especially in legumes (Bravo, 1998). Isoflavones (genistein) and coumestrans are the two classes of phyto-oestrogens of particular interest for human health. Phyto-oestrogens are naturally occurring plant chemicals (Mazur, 1998; Cassidy & Griffin, 1999) that are currently receiving considerable attention because of their potential in prevention of a range of hormone-dependent conditions, including cancer prevention, menopausal symptoms, coronary heart disease and osteoporosis. Phyto-oestrogens are structurally similar to oestrogen and therefore have the potential to mimic its effects *in vivo*. Isoflavones are present in high concentrations in soyabean products but in much lower amounts in most common pulses (Table 2). Coumestrans are measurable in most fibre-rich foods. The important coumestans include coumestrol, 4′-methyoxycoumestrol, sativol, trifoliol and repensol. The most abundant isoflavones are the glycosides of genistein (4′,7-dihydroxy isoflavone) and their 4-methyl ether derivatives, formononetin (7-hydroxy-4′-methoxy isoflavone) and Biochanin A (7-hydroxy-4′-methoxyisoflavone).

Tannins are compounds of intermediate to high molecular weight (up to 30 000 Da), which are highly hydroxylated and can form insoluble complexes with carbohydrates and proteins. The phenolic groups of tannins are bound to enzymes and other proteins by hydrogen binding to amide groups, and form insoluble tannin–protein complexes resistant to digestive enzymes of monogastric animals (Sosulski, 1979). This function of tannin is responsible for the astringency of tannin-rich foods (tea and some vegetables and fruits), especially when immature, because of the precipitation of salivary proteins. They can be subdivided into two major groups: hydrolysable and condensed tannins. Tannic acid, the best-known hydrolysable tannin, is a pentagalloyl glucose molecule that can further esterify with another five gallic units. Condensed tannins, or proanthocyanidins, are high molecular weight polymers. The monomeric unit is a flavan-3-ol (catechin, epicatechin, etc.). Oxidative condensation occurs between carbon C-4 of the heterocycle and carbons C-6 or C-8 of adjacent units. The most commonly described condensed tannins have molecular weights of approximately 5000 Da, although much larger molecules have been described in carob pods (*Leguminosae*) (Bravo, 1998).

Lignans are a group of diphenolic compounds with dibenzylbutane skeleton structures and characteristics similar to those of phyto-oestrogens.

The main polyphenolic compounds found in pulses are indicated in Table 3, i.e. mainly tannins, phenolic acids and flavonoids. Some data on the amounts of these compounds in pulses are given in Table 2.

The legumes with the highest polyphenolic content are the dark varieties, such as red kidney beans (*Phaseolus vulgaris*) and black gram (*Vigna mungo*). Condensed tannins (proanthocyanidins) have been quantified in hulls of several varieties of field beans (*Vicia faba*) and are also present in pea seeds of coloured-flowered cultivars (Savage, 1989; Gdala *et al.* 1992; Smulikowska *et al.* 2001). Tannin-free and sweet seeds have been selected among broad beans, lentils and lupins.

Pulses contain isoflavones, but their concentration in seeds is much lower than in soyabeans (Bravo, 1998). The soyabean is known to be a unique source of the isoflavones, genistein and daidzein.

### *Saponins*

Saponins are a diverse group of compounds commonly found in legumes, e.g. chickpeas, soyabeans, lentils, peanuts, *Phaseolus* beans and alfalfa sprouts; and in some plants commonly used as flavourings, herbs or spices (Oakenful & Sidhu, 1990). They are glycosides composed of a lipid-soluble aglycone consisting of a sterol or, more commonly, a triterpenoid structure attached to water-soluble sugar residues that differ in their type and amount. Many types of saponins can be present in the same bean.

Sojasapogenol B has been identified as the predominant sapogenol in lima beans and jackbeans (Oboh *et al.* 1998).

**Table 3.** Main polyphenolic compounds found in pulses

| | Basic skeleton | Examples |
|---|---|---|
| Phenolic acids | C6–C1 | Galic acid, syringic acid |
| Phenyl propanoids | C6–C3 | Coumarins, hydroxycinnamic acids (caffeic, ferulic, sinapic) |
| Flavonoids | C6–C3–C6 | Flavones (e.g. luteolin), flavonols (e.g. quercitin, kaempferol), flavanols, isoflavonoids (e.g. genistein, daidzein), proanthocyanidins (or condensed tannins) |
| Lignans, neolignans | $(C6–C3)_2$ | Secoisolariciresinol |
| Lignins | $(C6–C3)_n$ | |

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The saponin content of peas ranges from 1·1 g/kg for yellow peas to 2·5 g/kg for green peas, whereas the levels in lentils are 3·7–4·6 g/kg (Savage & Deo, 1989). The saponin contents of chickpeas and faba beans are apparently in the same range and lower than in soyabean (Table 2).

### *Alkaloids*

Alkaloids constitute a group of very diverse compounds that, in most cases, consist of a heterocycle with a nitrogen atom within the cycle. This conformation confers a basic character on the molecule, which tends to acquire a proton in aqueous solution, except when the nitrogen atom is close to an electron acceptor in the molecule (e.g. ricinine). They are mainly present in lupins, but breeding of alkaloid-free varieties ('sweet varieties') has increased the lupin content of fodder for all classes of domestic livestock (Bond & Duc, 1993).

Alkaloids are present in some other grain legumes, such as the jackbean in which trace quantities of lupanine have been found (Oboh *et al.* 1998).

### *Other compounds*

Cyanogenic glucosides are responsible for cassava toxicity, but can be found in some species of beans such as lima beans (especially black varieties), which can induce respiratory distress when eaten in large amounts (Montgomery, 1969). One of these compounds, phaseolunatin, produces cyanhydric acid and acetone after enzymatic hydrolysis (endogenous glucosidase). Cyanide-producing compounds are also found in much lower amounts in *Phaseolus vulgaris* and *Cicer arietinum*, less than 2 mg HCN yield/100 g as compared to 14–17 mg/100 g in most lima beans (*Phaseolus lunatus*) (Liener, 1979).

Vicine and convicine are glucosides (a molecule of glucose linked to a pyrimidine nucleoside structure) present in *Vicia faba* and known to be responsible for haemolytic anaemia (favism) in subjects with a glucose-6-phosphate dehydrogenase deficiency. Other grain legumes contain very small amounts of vicine and convicine as compared to faba beans (for instance, 21–49 μg/g in *Phaseolus vulgaris* as compared to 1480–2680 μg/g in *Vicia faba*; Saini, 1993).

Several toxic amino acids are present in some varieties of legumes (genera *Lathyrus* and *Vicia*; Roy, 1981). They are classified as neurotoxins (e.g. β-*N*-oxalyl-α,β-diaminopropionic acid), osteotoxins (e.g. β-aminopropionitrile) and antimetabolites (e.g. mimosine). The seeds can be detoxified by boiling and elimination of cooking water.

## Positive and negative effects of the main non-nutrient bioactive compounds of pulses

The so-called 'antinutrients' have adverse effects on animals when ingested regularly in large amounts over a long period of time. However, a number of potential health benefits have been identified during the past 10 years. Interestingly, these benefits appear to be similar to those suggested for dietary fibres in fruits, vegetables and grains, e.g. lower blood glucose and hormonal responses to starchy foods and a decrease of blood lipids and reduced cancer risk.

### *Enzyme inhibitors*

Protease inhibitors have been associated with growth inhibition and pancreatic hypertrophy in some experimental animals (Hathcock, 1991). The feeding of purified trypsin inhibitors or raw soya flour containing protease inhibitors can potentiate the effects of pancreatic carcinogens (Hathcock, 1991). However, these components can easily be denatured and inactivated by heat, although 5–20 % of their activity may still remain in commercially available soya products (Hathcock, 1991).

Protease inhibitors have been linked to pancreatic cancer in animal studies, but may also act as anticarcinogenic agents. Animal studies, *in vitro* cell culture work and epidemiological data have shown low cancer mortality rates in human populations with a high intake of protease inhibitors. *In vitro*, protease inhibitors can suppress the malignant transformation of cells induced by different types of carcinogens, e.g. ionizing radiation, ultraviolet light, chemical carcinogens and steroid hormones (Thompson, 1993; Clemente & Domoney, 2001). The most effective protease inhibitors have chymotrypsin inhibitor activity, such as those found in soyabean, chickpea and potato. The Bowman–Birk inhibitor derived from soyabean inhibited or prevented the development of chemically induced cancers of the liver, lung, colon, mouth and oesophagus in mice, rats and hamsters (Clemente & Domoney, 2001).

Protease inhibitors may act by several anticarcinogenic mechanisms, but their precise target is still unknown (Thompson, 1993; Clemente & Domoney, 2001).

Amylase inhibitors can reduce starch digestion, and some data suggest that they cause pancreatic hypertrophy. Amylase inhibitors derived from kidney beans did not affect the weight gain of rats (Savaiano *et al.* 1977), while white bean inhibitor retarded growth and caused liver and kidney changes (Maranesi *et al.* 1984). These conflicting data have been attributed to differences in pH sensitivity (Gallaher & Schneeman, 1986) and the sensitivity of pancreatic amylase to different amylase inhibitors (Ho *et al.* 1981).

Addition of amylase inhibitor reduced blood glucose and raised insulin levels after raw starch intake by rats, dogs and man (Puls & Keup, 1973), which suggests that this antinutrient could be used for therapeutic purposes in diabetes and obesity control. Several companies have marketed amylase inhibitor preparations for caloric control, referred to as 'starch blockers'. However, clinical studies showed that they did not affect post-prandial concentrations of plasma glucose and insulin and breath hydrogen (Carlson *et al.* 1983; Hollenbeck *et al.* 1983), possibly because of the low anti-amylase activity of the preparations. In fact, partial purification of amylase inhibitors blocked amylase activity *in vitro* and *in vivo* (Layer *et al.* 1985, 1986). Subsequently, reductions in post-prandial plasma glucose, insulin, C-peptide and gastric inhibitory polypeptide were observed when amylase inhibitors were given to healthy or diabetic individuals (Layer *et al.* 1986; Boivin *et al.* 1987, 1988). In man, the intake of

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non-purified preparations of 'starch blockers' caused gastrointestinal symptoms, e.g. diarrhoea, nausea and vomiting. This phenomenon was attributed to contaminants (Thompson, 1993).

### *Lectins or phytohaemagglutinins*

According to Liener (1989), lectins from most bean species are toxic, whereas some other lectins, e.g. from soyabeans, are not toxic. The toxicity of lectins is characterized by growth inhibition in experimental animals and diarrhoea, nausea, bloating and vomiting in man (Liener, 1989). Improperly cooked beans can be toxic for man, probably because of incomplete lectin denaturation (Noah *et al.* 1980; Bender, 1983).

Lectins may also influence the blood glucose response by binding to the intestinal mucosal cell, causing disruption and interference with nutrient absorption. Pusztai *et al.* (1998) concluded from studies on rats (Bardocz *et al.* 1996; Pusztai *et al.* 1998) that bean lectin could act as a therapeutic agent to stimulate gut function and ameliorate obesity if a safe and effective dose-range is established for human subjects.

Several papers from Pryme, Pusztai and Bardocz on various animal models (mice and rats) tend to show that phytohaemagglutinin from *Phaseolus vulgaris* limits tumour growth by promoting gut epithelium hyperplasia (Pryme *et al.* 1998, 1999).

### *Phytates and oxalates*

Phytic acid is very reactive with other positively charged ions, such as minerals (especially Zn, Ca and Fe), thereby forming insoluble complexes that are less available for digestion and absorption in the small intestine (Cheryan, 1980; Sandberg, 2002). Alternatively, the ability of phytic acid to chelate minerals may have protective effects, such as decreasing the risk of iron-mediated colon cancer and lowering serum cholesterol and triglycerides in experimental animals.

Phytic acid seems to have demonstrably effective anticancer action against a variety of experimental tumours. $IP_6$ has been effective on experimental mammary tumours (Shamsuddin & Vucenik, 1999), human prostate carcinoma cells (Zi *et al.* 2000), azoxymethane-treated rats (experimental colon carcinogenesis) (Reddy *et al.* 2000), 7,12-dimethyl benz(a)anthracene-treated mice (experimental skin carcinogenesis) (Ishikawa *et al.* 1999), and HepG2 cells (human liver cancer cell line) transplanted into nude mice (Vucenik *et al.* 1998*a*,*b*).

Oral administration of phytic acid inhibited colon carcinogenesis in rodents during the initiation and post-initiation stages (Reddy, 1999), and a similar effect was obtained by a single application of a carcinogen to animals receiving $IP_6$ in drinking water (Ishikawa *et al.* 1999).

In studies carried out to date, dietary phytic acid reduced the incidence of aberrant crypt foci (ACF) and putative preneoplastic lesions in rats. Phytic acid seems to act mainly as an antioxidant, reducing the rate of cell proliferation and augmenting the immune response by enhancing the activity of natural killer cells (Reddy, 1999). However, other mechanisms cannot be excluded (Thompson, 1993).

Phytic acid also seems to act as a lipid-lowering agent, as shown in different animal models: the Fischer rat model fed cholesterol-enriched or standard diets (Jariwalla, 1999) and rats fed on sucrose for 30 d (Katayama, 1995). The possible mechanisms of phytic acid action include its ability to bind to Zn and thus lower the plasma Zn:Cu ratio (lower ratios tend to predispose man to cardiovascular disease) (Klevay, 1977) or to reduce plasma glucose and insulin concentrations, which may lead to a reduced stimulus for hepatic lipid synthesis (Wolever, 1990).

Oxalic acid can also impair Ca absorption. In general, Ca absorption is inversely proportional to the oxalic content of food. A notable exception is soyabeans, which are rich in both oxalate and phytate, although soya products have relatively high Ca bioavailability. In contrast, common dried beans (*Phaseolus vulgaris*), which are also rich in phytate, have substantially lower Ca bioavailability (Weaver *et al.* 1993). Dietary oxalate is involved in the genesis of urinary calcium oxalate stones. In fact, mild hyperoxaluria is more important than hypercalciuria in the aetiology of Ca-containing renal stones (Marshall *et al.* 1972). It has been shown recently that a significant percentage of hypertensive and overweight subjects have a greater risk of renal stone formation. Higher oxaliuria appears to be one of the most important factors accounting for the greater risk in these patients (Borghi *et al.* 1999).

### *Phenolic compounds*

Most studies concerning the biological effects of phenolic compounds have been devoted to the physiological impact of flavonoids. However, two reviews (Thompson, 1993; Bravo, 1998) provide a general overview of the nutritional significance of all types of food polyphenols, including simple phenols, tannins and flavonoids.

The antinutritional and toxic effects of phenolic compounds, particularly tannins, have been categorized as: depression of food intake, formation of the less digestible tannin–dietary protein complexes, inhibition of digestive enzymes, increased excretion of endogenous protein, digestive tract malfunctions and toxicity of absorbed tannin or its metabolites (Jansman & Longstaff, 1993). Thompson (1993) also noted an increased risk of cancer of the mouth and oesophagus linked to dietary tannins in some cases, as observed in epidemiological studies. In animal nutrition, the adverse effect of tannins is generally attributed to their astringent taste, linked to precipitation of salivary proteins by solubilized tannins or binding with digestive enzymes or exogenous proteins (Jansman & Longstaff, 1993). The body may also develop certain defence mechanisms against tannins, including the induction of proline-rich, tannin-binding salivary proteins (Thompson, 1993).

Concern about the presence of dietary phyto-oestrogens was first apparent in the 1940s in relation to reports of infertility in sheep in Western Australia, which decimated the sheep-breeding industry (Bennets *et al.* 1946). This infertility syndrome, referred to as Clover disease, appears to have been caused by grazing in pastures with a high

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content of clover (*Trifolium subterraneum*), which is rich in phyto-oestrogens (Bradbury & White, 1954). Equol (4′,7-dihydroxyisoflavan), which is produced from phyto-oestrogens by colonic bacteria, is thought to have been responsible for the infertility (Lindsay & Kelly, 1970). As lignans show structural similarities to phyto-oestrogens, they are also considered to have oestrogenic and antifertility effects.

Phyto-oestrogens (isoflavones but also lignans) have the potential to mimic the effects of oestrogen *in vivo*. They are strikingly similar in chemical composition to oestradiol, bind to the oestrogen receptor, and produce typical, predictable oestrogenic responses in animals (Cassidy & Griffin, 1999). However, on a molar basis relative to physiological oestrogens, isoflavones are quite weak, possessing between $1 \times 10^{-4}$ and $1 \times 10^{-3}$ the activity of 17β-oestradiol (Messina, 1999). They offer potential alternative therapies for a range of hormone-dependent conditions, including cancer prevention, menopausal symptoms, coronary heart diseases and osteoporosis.

Controlled intervention studies in pre-menopausal women provide direct evidence suggesting that diets containing phyto-oestrogens can produce oestrogenic effects in women of reproductive age (Cassidy *et al.* 1994, 1995). Phyto-oestrogens can also act as a weak oestrogen in post-menopausal women (Cassidy *et al.* 1998) and have the potential to exert similar effects to those of hormone replacement therapy. This is supported by epidemiological data on the incidence of coronary heart diseases in Asia versus Western countries, but also in vegetarians (Cassidy & Griffin, 1999). However, this protective effect can also be attributed to other components, such as antioxidant micronutrients.

The role of phyto-oestrogens in the hypocholesterolaemic effect of soyabeans is not proven, as no randomized crossover trials have been conducted to examine the effect of phyto-oestrogen-rich diets on the spectrum of biochemical markers of lipoproteins and haemostatic factors in human subjects (Cassidy & Griffin, 1999). However, purified isoflavone supplements do not seem to affect the lipid profile (Nestel *et al.* 1997; Hodgson *et al.* 1998). Further studies need to be conducted on hypercholesterolaemic subjects using phyto-oestrogen-rich foods and a wide range of biochemical markers of coronary heart disease risk (Cassidy & Griffin, 1999). Anderson *et al.* (1995) suggested that isoflavone may account for up to 60 % of the hypocholesterolaemic effect of soyabean, but obviously other mechanisms can be involved, as soyabean foods low in phyto-oestrogens also have cholesterol-lowering effects (Sirtori *et al.* 1997). Genistein (an isoflavone precursor) can inhibit (*in vitro*) endothelial cell proliferation, migration and tube formation, as well as thrombin formation and platelet activation (Sargeant *et al.* 1993; Wilcox & Blumenthal, 1995). This same compound has been found in the urine of healthy human subjects consuming a soyabean-based vegetarian diet (Fotsis *et al.* 1993). It also enhanced the resistance of LDL to oxidation *in vitro* and was the most potent isoflavone antioxidant (Ruiz Larrea *et al.* 1997). Therefore, genistein may slow the development of atherosclerotic disease.

Phenolic compounds such as ferulic and *p*-coumaric acid, at the low levels found in Bengal gram, lowered blood lipid levels in rats and are thus thought to contribute to the hypocholesterolaemic effect of Bengal gram (Sharma, 1980, 1984).

It is considered that phyto-oestrogens, quercitin (flavonol) and lignans may enhance tumour growth, as oestrogens have growth-stimulatory effects (Miller, 1990). Although their aromatic structure suggests that they may also be carcinogenic, this has not been confirmed. On the contrary, epidemiological data and the biological properties of phyto-oestrogens (isoflavones and lignans) suggest that they may be important in the prevention and control of hormone-dependent cancers. All soya protein products consumed by Asian populations have high concentrations of isoflavonoids, and Japanese women on traditional diets seem to have a lower risk of breast cancer (Adlercreutz *et al.* 1988). In other countries, such as Finland and Sweden, lignan levels are higher in populations with the lowest cancer risk because of high consumption of wholegrain rye bread, berries and some vegetables. Breast cancer has been found to be associated with low lignan levels in the USA, Finland, Sweden and Australia. Evidence concerning the effect of phyto-oestrogens on prostate and colon cancer seems to be very limited. However, the risk of prostate cancer is much lower in Asian than in Western men. This lower risk has been associated with the higher consumption of isoflavones in Asia than in the West, i.e. 20 mg/d in the Japanese male eating traditional food and less than 1 mg/d in Western men. This is reflected by the respective plasma genistein concentrations: 180 ng/ml ($n = 72$) in Japanese men versus $< 10$ ng/ml in Western males (Griffiths *et al.* 1999). Another recent study showed an inverse association between coumestrol ($P = 0{\cdot}03$) and diadzein ($P = 0{\cdot}07$) and the risk of prostate cancer in the Caucasian population (Strom *et al.* 1999).

In azoxymethane-treated rats, soya protein (rich in isoflavones), as compared to a control group, increased the number of small ACF, whereas rye bran (rich in lignans) decreased the number of large ACF, indicating that rye may be more favourable than soya products for colon tumour prevention (Davies *et al.* 1999). Quercitin and rutin, two flavonoids, reduced azoxymethane-induced colon cancer in mice (focal areas of displasia), but surprisingly, quercitin alone was able to induce focal areas of dysplasia in 22 % of normal mice fed a standard diet (Yang *et al.* 2000).

Phyto-oestrogens (isoflavonoids and lignans) may have positive effects on cancer risk through their oestrogenic activity, but also by interfering with steroid metabolism and bioavailability. They may also inhibit enzymes, such as tyrosine-specific protein kinase and DNA topoisomerase (Markovits *et al.* 1989), which are crucial to cell proliferation (Denis *et al.* 1999). Concentrations of plasma insulin-like growth factor I, which is associated with increased breast cancer risk, may be involved in the anticancer effect of linseed, as shown in a rat model of mammary tumour (Rickard *et al.* 2000).

The chemopreventive potential of some phyto-oestrogens may be different between males and females, as suggested by a recent *in vitro* study on male and female pancreatic tumour cells (Lyn-Cook *et al.* 1999).

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Several phenolics (e.g. chlorogenic acid, gallic acid, caffeic acid, tannic acid and catechin) can also inhibit the mutagenic effects of both direct-acting carcinogens (e.g. benzo(a)pyrene diol epoxide) and carcinogens that require metabolic activation (e.g. aflatoxin $B_1$), and trap nitrite, thereby reducing nitrosating species and preventing endogenous formation of carcinogenic nitrosamines.

### *Saponins*

As saponins are poorly absorbed, most of their effects are probably attributable to their hydrophilic/hydrophobic asymmetry and consequently their capacity to reduce superficial tension. Erythrocytes are disrupted in saponin solutions due to interactions with cholesterol in the erythrocyte membrane (Birk & Peri, 1980). This characteristic explains why saponins are acutely toxic when injected intravenously. They confer a bitter taste on food, so that some plants are not eaten by animals (Birk & Peri, 1980). Most saponins form insoluble complexes with 3-β-hydroxysteroids and are known to interact with, and form large mixed micelles with, bile acids and cholesterol. Saponins have been studied most extensively for their hypocholesterolaemic effect, but their long-term toxicity for humans is unknown (Thompson, 1993). Decreased cholesterol has been observed in numerous animal species (chickens, rats, mice or monkeys) fed diets containing saponins from fenugreek (Sharma, 1986), soya (Oakenful *et al.* 1984) and chickpea (Malinow *et al.* 1977) in the presence or absence of cholesterol (Rao & Kendall, 1986). Although evidence for the hypocholesterolaemic effect of saponins is very strong in animals, particularly those fed in the presence of cholesterol, the results for human studies are less conclusive (Milgate & Roberts, 1995). However, according to Thompson (1993), diets containing foods rich in saponins (300–500 mg/d), such as soya, chickpea and bean meal, can reduce plasma cholesterol by 16–24 %. Saponins may lower cholesterol by binding to dietary cholesterol (Gestener *et al.* 1972) and preventing its absorption and/or by binding to bile acids and thereby interfering with its enterohepatic circulation and increasing its faecal excretion (Sidhu & Oakenful, 1986). Increased bile acid excretion, by causing a compensatory increase in bile acid synthesis from cholesterol in the liver, lowers plasma cholesterol. It has recently been shown that ginseng saponins decrease serum cholesterol and triglycerides in rats and rabbits by sustaining lipoprotein lipase at a normal level or protecting LPL activity from being decreased by several factors, including chemically induced hyperlipidaemia (in rabbits) or a high glycerol/fructose diet (in rats; Inoue *et al.* 1999).

The biological properties of saponins suggest that they may also have some anticarcinogenic effects (Messina, 1999). A rodent study found that a saponin-containing diet (3 % by wt) inhibited the development of azoxymethane-induced preneoplastic lesions in the colon by about two-thirds (Koratkar & Rao, 1997). However, human intake of saponins is estimated to be generally $<$200–300 mg/d for a total food intake of approximately 500 g (dry weight) (Ridout *et al.* 1988).

**Table 4.** Main non-nutrient bioactive substances present in legumes and processes commonly used for their reduction or elimination (adapted from Frøkiær *et al.* 2001)

| Bioactive substance | Commonly used elimination processes |
|---|---|
| Enzyme inhibitors | Heat treatment<br>Fermentation |
| Lectins | Heat treatment |
| Phytic acid | Enzymatic degradation (exogen/endogen phytase), germination, fermentation |
| Oxalate | Cooking, dehulling |
| Phenolic compounds | Dehulling |
| Saponins (soya) | Sprouting |

## Elimination of minor bioactive substances by technological treatments

A number of treatments of grain legumes are able to eliminate some bioactive substances partially or totally (Table 4), including soaking, dry and moist heat treatment, filtration, germination, fermentation and enzymatic treatments. Obviously, chemical and physical characteristics determine the choice of appropriate treatment used to eliminate an undesirable compound from food.

### *Enzyme inhibitors*

Trypsin inhibitors, which are important in domestic livestock nutrition (Huisman, 1990), may be rather unimportant in cooked pulses, as they are easily heat-labile (even though 5–20 % of the activity may still remain in commercially available soya products) (Hathcock, 1991). The feeding of purified trypsin inhibitors or raw soya flour containing protease inhibitors can potentiate the effects of pancreatic carcinogens (Hathcock, 1991).

About two-thirds of the haemagglutinating and antitrypsin activity of *Vicia faba* L. and *Pisum sativum* can be eliminated during wet fractionation and preparation of protein isolates. The amount of antitrypsin factor can be further lowered by subjecting the protein extract to ultrafiltration (Gueguen *et al.* 1980).

### *Lectins or phytohaemagglutinins*

Heat processing can denature lectins and reduce their toxicity, but a low temperature or slow cooking may not be sufficient to eliminate toxicity completely (Thompson *et al.* 1983). The work of de Muelenaere (1965) in South Africa drew attention to the real risks from residual lectins. Continuing vigilance is required, as some bean varieties may have much higher lectin content than others, and residual quantities of the initial levels may resist even normal cooking at altitudes well above sea level (de Muelenaere, 1965).

### *Phytates and oxalates*

The phytic acid content of pulses is significantly decreased by germination (Chitra *et al.* 1996; Vidal-Valverde *et al.* 2001) and to a lesser extent by fermentation (Chitra *et al.* 1996). Heat treatments such as autoclaving or roasting also

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seem to reduce the phytic acid content of pulses, although it cannot be excluded that such apparent decreases are artifacts due to a decrease of phytic acid extractability (Chitra *et al.* 1996).

Gad *et al.* (1982) reported that both cooking and dehulling beans reduced the oxalate content of the grain.

### *Phenolic compounds*

The level of polyphenols in plant foods is largely influenced by genetic factors and environmental conditions. Other factors, such as germination, degree of ripeness, variety, processing and storage, also influence the content of plant phenolics (Bravo, 1998). Many studies have contributed to tannin reduction by selecting for white-flowering low-tannin cultivars or dehulling the seeds of colour-flowering high-tannin cultivars, to improve the digestibility of field beans for animals, mainly pigs (Longstaff and McNab, 1991). Bressani and collaborators pointed out long ago the differences in the digestibility of Central American beans relative to differences in the 'tannins' of their testas (Fukuda *et al.* 1982).

## Conclusions

On the one hand, so-called 'antinutrients' have adverse effects, mainly demonstrated in extreme situations, such as experiments on animals involving high levels of grain legumes (usually distributed without any major technological treatments, except grinding) or even high levels of purified 'antinutrients'. On the other hand, the beneficial impact of these same compounds has been shown in similar situations, but in most cases in relatively short-term studies. Interestingly, the health benefits appear to be similar to those suggested for dietary fibres in fruits, vegetables and grains, i.e. lower blood glucose and hormonal responses to starchy foods and a decrease in blood lipids and reduced cancer risks.

The main adverse effects of the non-nutrient bioactive substances of pulses that could cause problems in human nutrition are antinutritional effects characteristic of insufficiently denatured enzyme inhibitors and lectin, which can occur in populations with a lack of energy sources suitable for cooking foods properly.

The following are the main benefits of the minor bioactive substances of pulses:

- The anticarcinogenic properties of protease inhibitors, phytic acid, phyto-oestrogens and lignans, saponins and phenolic compounds.
- The decrease of blood glucose (and insulin) response attributed to phytic acid, lectins, amylase inhibitor or polyphenol compounds (or tannins), as well as the role of the starch and dietary fibre present in large amounts in pulses.
- The hypolipaemic effect attributed to saponins, phytosterols, isoflavones and phytic acid, as well as the possible role of dietary fibre and proteins (Dubois *et al.* 1993; Alonso *et al.* 2001).

The overall beneficial effect of pulses for human health, when consumed in significant amounts, is attributable to their macronutrient composition, although it is likely that the non-nutrient bioactive substances present in the pulses play a role. Therefore, it is probably not suitable to remove all these substances systematically by technological and especially plant breeding means. However, a systematic elimination may be needed when incorporation of pulses, or the pulse fraction, is increased in baby foods, or when a specific type of pulse is consumed at a very high level by one group of the population.

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