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Phytochemicals for Health, the Role of Pulses

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Pulses are the seeds of legumes that are used for human consumption and include peas, beans, lentils, chickpeas, and fava beans. Pulses are an important source of macronutrients, containing almost twice the amount of protein compared to cereal grains. In addition to being a source of macronutrients and minerals, pulses also contain plant secondary metabolites that are increasingly being recognised for their potential benefits for human health. The best-studied legume is the soybean, traditionally regarded as an oilseed crop rather than a pulse. The potential health benefits of soy, particularly with respect to isoflavone content, have been the subject of much research and the focus of several reviews. By comparison, less is known about pulses. This review investigates the health potential of pulses, examining the bioactivity of pulse isoflavones, phytosterols, resistant starch, bioactive carbohydrates, alkaloids and saponins. The evidence for health properties is considered, as is the effect of processing and cooking on these potentially beneficial phytochemicals.

KEYWORDS: Pulses; phytochemicals; macronutrients

INTRODUCTION

Pulses have traditionally played a major role in providing food nutrition particularly in the Indian subcontinent and other developing countries, while in western countries, the staple diet has been based on animal-derived protein.

Traditionally, pulses were consumed with minimal processing, and consumers were interested primarily with size, shape, and color characteristics. The markets were driven by price and availability. As the countries of the Indian subcontinent developed, a greater emphasis was placed on processing characteristics, which included hydration and cooking times as well as dehulling and splitting efficiency. This represents the current status for the majority of markets that consume pulse grains as a staple diet. While these market traits represent basic quality characteristics, the underlying chemical characteristics are based on protein and starch composition and phenolic compounds that affect the taste and color of the seed coat and cotyledon.

The nutritional properties of pulses have been investigated extensively and have been reported to impart physiologically beneficial effects in humans. Pulse grains are high in protein, carbohydrates, and dietary fiber and are a rich source of other nutritional components (*1*). The chemical composition and nutritive value of Australian pulses has been collated by Peterson et al. (*2*).

The value of pulses can be enhanced by physically fractionating the grain into basic constituents such as protein, starch, and fiber and using these products to supplement other food ingredients to enhance the nutritive value of food. There is now an increased awareness of the health-associated value of pulses in western countries. Pulse grains contain a large number of bioactive compounds which have a metabolic benefit when consumed on a regular basis (*3*).

Demand has increased regarding the use of pulses for human consumption either to extract a functional compound (e.g., starch protein or fiber) to incorporate this into cereal-based products or to extract phytocompounds which are bioactive and can be used as nutraceutical products.

Figure 1 represents the changing emphasis for plant breeding and consumer demand. There is a need to increase the knowledge base for pulses by understanding more of the functional and bioactive properties of pulse grains (*4*).

Considerable genetic variation has been reported in the chemical composition of pulses both between and within species. In addition, chemical composition is modified by environmental factors during plant development, and many of the phytocompounds are secondary metabolites produced during seed development and seed maturation.

This paper reviews the current knowledge around certain classes of pulse phytochemicals, including starch, phytosterols,

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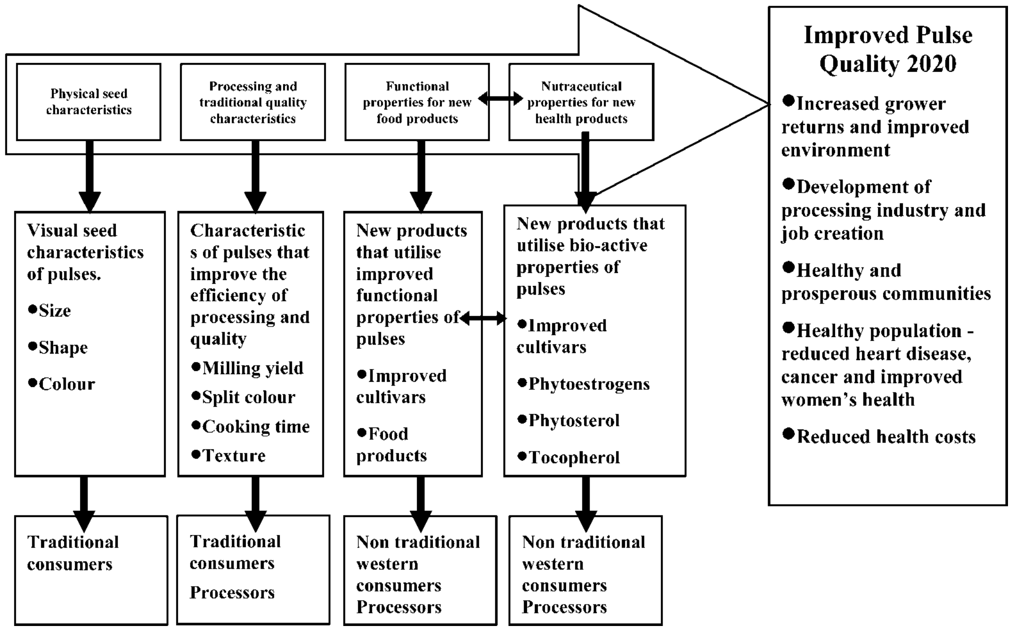


Figure 1. Developing quality pulses for a sustainable environment, population, and community. (*4*)

isoflavones, saponins, alkaloids, and bioactive carbohydrates. The potential for these metabolites to influence human health is discussed as are processing methods and agricultural practices that influence the levels of these compounds in food.

RESISTANT STARCH

Starch is a major carbohydrate in pulse grains, and due to its high concentration of amylose, the process of digestion and metabolism is therefore of interest, particularly as there is a strong negative correlation between the intake of starch and the risk of colorectal cancer (*5*).

Starch can be classified according to digestibility as soluble, insoluble, or resistant starch (RS). Until recently, starch was thought to undergo complete breakdown and absorption upon digestion. In 1992, Englyst et al. referred to RS as the proportion of starch that is not hydrolyzed or digested as it passes through the gastrointestinal tract (*6*). Resistant starch that reaches the large intestine has a physiological function similar to that of dietary fiber. Resistant starch can be considered a probiotic and acts as a substrate for microbiological fermentation, producing short-chain fatty acids (SCFAs), methane, and carbon dioxide, conferring benefits to human colonic health, and to a lesser extent can impact lipid and glucose metabolism. The production of these fermentation products from the consumption of RS is less than that from the consumption of nondigestible oligosaccharides (*7*). It is believed that the SCFAs produced mediate the benefits of RS rather than RS exerting a physical bulking effect (*8*).

Short-chain fatty acids consist principally of butyrate, propionate, and acetate and are metabolic products of anaerobic bacterial fermentation (*9*) and are the preferred respiratory fuel of the colonocytes lining the colon. These cells serve to increase blood flow, lower luminal pH, and help prevent abnormal colonic cell populations (*10*). Human feeding studies have shown that RS consumption in a diet results in an increase of SCFA in the colon (*11*).

Pulse grains are high in RS (**Table 1**) and retain their functionality even after cooking (*12*).

Worldwide, the dietary intake of RS varies considerably. In developing countries, the intake is between 30 and 40 g/day (*13*). In the EU, the intake of RS is between 3 and 6 g/day (*14*), and in Australia, a similar intake has been reported by Baghurst et al. (*13*) These figures represent the total amount from all sources including fruit and vegetables.

Table 1. RS Composition in Pulse Grains and Wheat Bran (*12*)

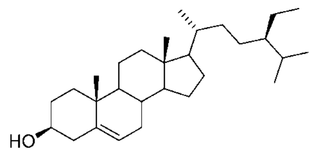
grain product	raw (% RS)	cooked (% RS)
field pea (*229*)	2.4	1.9
lentil (*229*)	3.3	2.5
chickpea (*229*)	3.4	2.3
wheat (bran)	0.4	not reported

Heat treatment or cooking of pulses increases hydrolysis; however, incomplete starch gelatinization and the formation of RS induced by high amylose starch results in lower digestibility (*15*) and may contribute to low glycemic responses in humans (*6*). As a food ingredient, RS has a lower calorific (8 kJ/g) value compared with fully digestible starch (15 kJ/g); however, it can be incorporated into a wide range of mainstream food products such as baked products without affecting the processing properties or the overall appearance and taste of the product (*16*). This may represent an opportunity to increase the consumption of pulse grains by fractionating pulses and incorporating these products into cereal-based products.

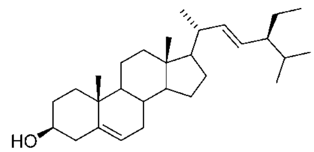
PHYTOSTEROLS

Epidemiological data indicates that the consumption of grains, including pulses, lowers the mortality rates from cardiovascular disease (*17*). Elevated levels of serum low-density lipoprotein (LDL) cholesterol is a major cause of cardiovascular disease, and studies have shown that every reduction of 1% in LDL cholesterol results in a 1% reduction in cardiovascular mortality (*18*). These and other studies have led to the promotion of lifestyle changes, which have resulted in a reduction in LDL cholesterol.

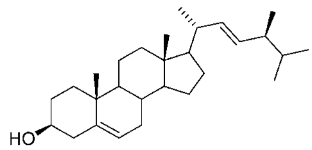
The consumption of pulse grains has been reported to lower serum cholesterol and increase the saturation levels of cholesterol in the bile. A dietary study conducted by Duane on humans over a seven week period showed that serum LDL cholesterol was significantly lower during the consumption of a diet consisting of beans, lentils, and field peas (*19*). The study showed that consumption of pulses lowers LDL cholesterol by



[1] β-sitosterol

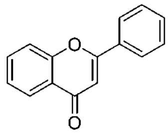


[2] campesterol

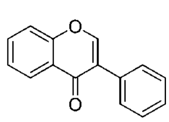


[3] stigmasterol

Figure 2. Common phytosterols.



Flavone



Isoflavone

Figure 3. Generic structure of flavone and isoflavone classes.

partially interrupting the entrohepatic circulation of the bile acids and increasing the cholesterol saturation by increasing the hepatic secretion of cholesterol. The study also concluded that other pulse components in the diet may also have contributed to the observed effect; in particular, saponins, which are hydrolyzed by intestinal bacteria to diosgenin, may have exerted a positive effect (*20, 21*). Several studies have demonstrated the efficacy of plant sterols and stanols in the reduction of blood cholesterol levels, and plant sterols are increasingly incorporated into foods for this purpose (*22, 23*).

Phytosterols are structural components of the plant-cell membranes. In pulses, they are present in small quantities, and the most common phytosterols are β-sitosterol (**1**), campersterol (**2**), and stigmasterol (**3**), **Figure 2** (*24*). These compounds are also abundant as sterol glucosides and esterified sterol glucosides, with β-sitosterol representing 83% of the glycolipids in defatted chickpea flour (*25*).

ISOFLAVONES

Flavones and isoflavones have been isolated from a wide variety of plants, though the isoflavones are largely reported from the Fabaceae/Leguminosae family. There has been enormous interest in their biological activity. Chemically, they are based on phenylchromen-4-one and have the general structures shown in **Figure 3**. The increased interest in the biological effects of these molecules can be demonstrated by the increase in published literature in this area. **Figure 4** graphs the number of references citing biological activity of flavones or isoflavones since 1940. The number of publications in the past few years (2000 on) is more than double that published in the entire preceding 60 years (1263 vs 619). Of these, 570 papers deal specifically with compounds from legumes.

In terms of specific compounds studied, genistein and daidzein are the most cited (275 and 189 references, respectively) with the related glycosides, genistin and daidzin (54 and 46 references, respectively) also the subject of considerable interest. Five of the most reported isoflavones are genistein (**4**), daidzein (**5**), coumestrol (**6**), formononetin (**7**), and biochanin A (**8**) (**Figure 5**). Genistin and daidzin are the seven-glucose derivatives of **4** and **5**, respectively.

According to the USDA survey on isoflavone content, lentils do not contain significant amounts of these isoflavones (*26*).

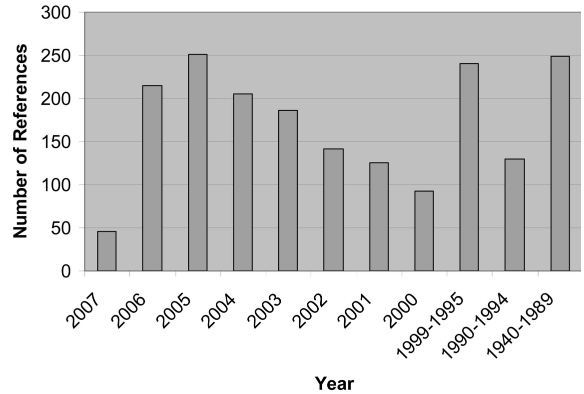
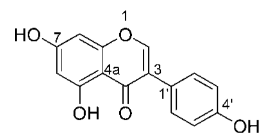
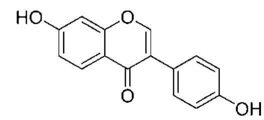


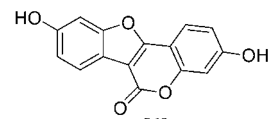
Figure 4. References reporting biological activity for flavones or isoflavones (search terms: Scifinder (all databases selected) using the phrase "activity of isoflavone or isoflavonol or isoflavanol" on April 23, 2007).



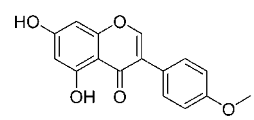
[4]



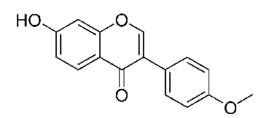
[5]



[6]



[7]



[8]

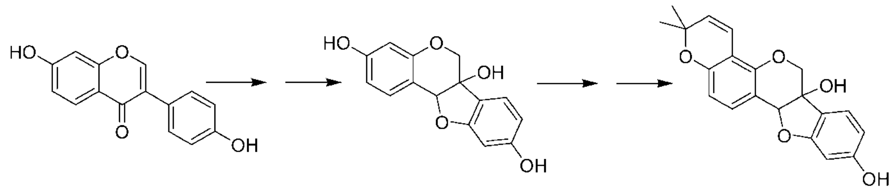
Figure 5. Important isoflavones.

Chickpeas contain 0.04 mg/100 g daidzein, 0.06 mg/100 g genistein, 0.14 mg/100 g formononetin, and approximately 1.7 mg/100 g biochanin A. Soybeans have significantly higher levels of daidzein (47 mg/100 g) and genistein (74 mg/100 g) but contain less formononetin and biochanin A compared to chickpeas, 0.03 mg/100 g and 0.07 mg/100 g, respectively. No figures are given for lupins.

Recently, there has been attention focused on a different class of isoflavones, the glyceollins, which have been reported from soy. These are biosynthetically related to pterocarpan and probably derive from the condensation of pterocarpan and a C5-terpene, **Figure 6** (*27*). The glyceollin types of isoflavone have not been reported from chickpeas, lupins, or lentils, despite the fact that chickpeas are capable of synthesizing the related, upstream metabolite daidzein.

Activities and Bioavailability. There are many biological activities associated with the isoflavones, including a reduction in osteoporosis and the prevention of cancer and cardiovascular disease, and they can be used for the treatment of symptoms of menopause. The potential health benefits of isoflavones for humans have been the subject of several reviews that have analyzed clinical, animal, and in vitro evidence for biological activity (*28–41*).

Since the early 1990s, a significant research effort has focused on the putative anticancer effects of isoflavones, in particular, the effect on breast cancer. Initial interest in this area was due to epidemiological observations of low breast cancer occurrence in Asian populations where the intake of soy and associated isoflavones is high. There has been sufficient research in this area that, in November 2005, a workshop was held to review



[5] daidzein [9] pterocarpan [10] glyceollin

Figure 6. Biosynthetic relationship between isoflavones found in soy.

the literature and make research recommendations (*32*). The recent meta-analysis by Trock et al. (*28*) highlights the difficulties of comparing literature clinical studies. The authors studied 12 case-control and six cohort studies (with the number of subjects varying from 88 to 1459) but were forced to make several assumptions regarding the quantities of isoflavones ingested. Trock et al. conclude there is a small inverse correlation between soy intake and breast cancer but note that data limitations cannot rule out the possibility that this result is an artifact of the analysis. One of the recommendations of the conference was to encourage future studies to reduce the heterogeneity of soy exposure data in the literature and provide more detail regarding not only total soy food but also nutrient content, such as the levels of isoflavones (*29*).

The intake of isoflavones has been recommended for menopausal women to relieve symptoms of menopause (instead of hormonal replacement therapy, HRT) (*42*). A recent review by Cassidy et al. (*34*) concluded that, although additional studies are required, there was limited evidence for the ability of isoflavones to relieve the symptoms of menopause such as hot flashes. The HRT-like actions of the isoflavones are thought to be due to the estrogenic effects of the metabolites. Recently, the wisdom of the recommendation to increase isoflavones for their HRT effect has been challenged due to the potential increased risk of breast cancer in those using HRT, with some in vivo and vitro work suggesting genistein and daidzein may stimulate estrogen-dependent human breast tumor growth (*43–45*). The isoflavones display both estrogen agonist and antagonist activity (*46*). Again, the evidence here is confounding, with conflicting results in the literature, but the recent study by Wood et al. (*46*) suggests that, in part, this may be due to differences in endogenous estrogen levels in the reported studies. The authors found no estrogenic effect for isoflavones at low levels of estrogen, as would be the case in menopausal women, a different situation to that of the various rat models where there is no basal level of estrogen. Studies also show that the isoflavones bind preferentially to the β form of the estrogen receptor (ERβ) rather than ERα (*47*). It is generally thought that the estrogen-associated risk of breast cancer is modulated through the ERα isoform. The ERα isoform promotes epithelial proliferation in the breast, while ERβ does not (*48*). Although genistein and daidzein have received the most attention in this area, there is also evidence that the glyceollins act through the ER pathways (*49, 50*).

Interestingly, there is also some evidence that the degree of processing may have an impact on the ability to stimulate tumorogenisis, with highly processed products more likely to be problematic (*45, 51, 52*). This conclusion would seem to correlate with the epidemiological evidence of a reduced risk of breast cancer in Asian populations, where the soy products are minimally processed. It has also been suggested that early and prolonged exposure to isoflavones, through a high-legume diet, is more beneficial than a later, higher consumption of isoflavone supplements (*45*).

Mechanistic studies suggest that the isoflavones may promote cancer cell death not only through ERβ but also more directly through the down-regulation of cell survival enzymes such as NF-kappaB (*53–56*), the activation of apoptosis via ER stress pathways (m-calpain, GADD153, GRP78, and caspase-12) (*57, 58*), and mitochondrial apoptotic pathways (Mcl-1 down-regulation and Bad cleavage) (*57*). These observations suggest that the isoflavones have the potential to prevent other cancers as well, and there have been studies around prostate cancer (*53, 59–67*), colorectal cancer (*68, 69*), and head and neck cancer (*54*). The research includes in vitro, in vivo, and clinical studies, and as with breast cancer, the results of these studies are mixed, but indicative of some protective effect due to isoflavones.

One of the potential confounding factors in cohort studies is the possibility that studies of single nutrients and food may be inconsistent because they do not account for related foods or the potential synergistic interaction of food combinations and other factors that may effect bioavailability (including cooking). In an effort to address these, Velie et al. (*70*) undertook a large diet-based cohort study (40 559 postmenopausal women). They found three diet groups across the U.S.A.; the only diet with significant negative correlation with invasive breast cancer was the traditional southern diet, which correlated to high legume intake, low mayonnaise intake, and potentially cabbage intake. This "whole of diet" or "whole of food" approach may indeed be a very important consideration since, as discussed later, the legumes that contain these isoflavones also contain other metabolites (in particular, saponins and sugar derivatives) which also possess anticancer activity.

Pharmacokinetic studies indicate that the plasma levels of the isoflavones can reach biologically significant levels (low micromolar) (*46, 71*). The glycosides are hydrolyzed to produce the aglycones, which have a half-life in the plasma of 4–8 h (*71*). Interestingly, equol (*11*) a human metabolite of daidzein, which is also highly bioactive, is not found ubiquitously. In a recent study, this metabolite was found in only 30% of women studied. Equol is likely to be a product of action by intestinal microflora, and it has been suggested that it may be more bioactive than the parental isoflavone (*36*). This observation introduces yet another potential source of variability in clinical studies—since an individual's microflora may be highly specific, the products of gut bacteria will vary between subjects in a study, and this may have large contributing effects on the intersubject variability.

There is growing evidence that the isoflavones may have a role to play in the treatment of metabolic disorders. A meta-analysis of 38 different controlled clinical trials concluded that soy protein intake led to decreased serum concentrations of total cholesterol, LDL cholesterol, and triglycerides (*35*). Studies in rats have shown that chickpea consumption can normalize triacylglyceride levels in hypercholesterolemic rats (*72*). This area of research is in its infancy compared to the enormous focus that isoflavones and cancer have received; however, there have been several mechanistic studies which suggest potential efficiency. Some of this action is related to the compounds' ability to act in a similar way to estrogen. Genistein, daidzein, and biochanin A have been reported as estrogen-related receptor α (ERRα) agonists. The orphan ERRs comprising ERRα, ERRβ, and ERRγ bind and regulate transcription via estrogen response elements but do not bind endogenous estrogens. ERRα is involved in energy homeostasis and so is a likely target for the treatment of metabolic disorders (*73*). PPARα and PPARγ (the peroxisome proliferator-activated receptors) active compounds are used to correct dyslipidemia and to restore glycaemia balance, respectively. Formononetin, biochanin A, genistein, and daidzein act as PPARα and PPARγ activators. Biochanin A and formononetin, in particular, are of interest in that they are active at low doses (1–4 μmol/L) (*74*). These compounds are both present in greater amounts in chickpeas compared to soy beans, and for conditions such as type II diabetes, the intake of pulses such as chickpeas may be of greater benefit.

Even less well-studied is the potential protective effect of isoflavones against neurodegenerative diseases. Although much more experimental evidence is required to investigate this hypothesis, the initial reports are intriguing. Interest in this area again arose from epidemiological studies that suggested post-menopausal women using estrogen replacement therapy had a decreased risk of developing dementia (*75, 76*). Genistein, daidzein, and glycitein (*12*) were examined in a transgenic nematode model for their ability to alleviate β amyloid expression-induced paralysis. Only glycitein demonstrated significant activity, and this at a relatively high concentration (100 μg/mL) (*77*). However, the ability of this isoflavone to reduce the formation of the β amyloid is nonetheless fascinating and surely warrants further investigation. More recently, an investigation of biochanin A suggested it may be protective against Parkinson's disease through its ability to protect dopaminergic neurons (*78*). These intriguing results suggest that pulses may have a role to play in healthy aging strategies, though clearly much additional research is required.

Cooking/Processing Effects. Although there have been several studies on pulses investigating the effect of cooking techniques on the removal of compounds such as phytate, oligosaccharides, and saponins (as discussed later), these studies have not, in general, addressed the stability of the isoflavones. There have been several studies on the isoflavone content of foods (*26, 79–83*) but few tracing their stability from the legume to the processed product. One of the more detailed reports in this area demonstrated that processing does have a significant effect on isoflavone content and indeed causes chemical modification of the isoflavones. The most common observation was the loss of the esterified malonate to form the glycoside of the isoflavones under any heat conditions (including baking and frying) (*84*). These authors also noted the almost complete absence of any isoflavones in the "low-fat" soy products. On the basis of the published literature, it seems the greater the degree of processing, the lower the amounts of isoflavones in the resulting product, but it remains unclear actually how much of the bioactives are lost during different processing methods.

Agricultural Studies. There is good evidence that farming practice can directly influence the levels of isoflavones in crops. The majority of this work has focused on soy, and it is likely that the results would transfer to pulses, though this hypothesis requires testing. One field study on soy beans demonstrated that irrigation enhances isoflavone content by as much as 2.5 fold (*85*). The application of potassium-rich fertilizer also results in an increase in the desirable bioactives (*86*). A 3-year breeding study demonstrated that levels of isoflavones are related to both environmental and genetic elements that would be susceptible to selection (*83*), and these genomic regions have been identified (*87*). This is an active area of research; for example, the U.S. Agricultural Research Service has initiated research projects working to understand the elicitation mechanism of flavones to enhance content in plants (*88*).

The isoflavones of edible legumes are not well-characterized for their natural role in plants, but there is evidence that many are antimicrobial and may have a role in plant protection. For example, the antifungal activity of lupin isoflavones has been demonstrated (*89*). In a study on the soybean cultivar response to fungal attack, it was noted that of two saprophytic fungi (*Mucor ramosissimus* and *Rhizopus* sp.) only *M. ramosissimus* induced an accumulation of metabolites including isoflavones. In the strains resistant to the fungus, a greater number of isoflavones (including glyceollins I, II, and III; glycinol; glyceocarpin; genistein; isoformononetin; and N-acetyltyramine) were induced, and with the exception of genistein, the compounds were demonstrated to possess antifungal activity (*90*). The cell-wall glucan from another fungus (*Phytophthora megasperma Drechs. f. sp. glycinea*, Kuan and Erwin) is also an elicitor of protective isoflavones (including the glycoside conjugates) in the soybean (*91*). An investigation of the fungal elicititor from *Diaporthe phaseolorum f. sp. Meridionalis*, the causal agent of stem canker, suggested that the elicitation of isoflavones may be mediated by the nitric oxide synthase pathway (*92*).

Interestingly, there is some evidence that specific symbiotic interactions have evolved to take advantage of the isoflavones' chemistry. It has been demonstrated that the symbiotic relationship between the fungus *Rhizobium lupini* and *Lupinus albus* stimulates an increase in production of prenylated isoflavones in the root nodules (*93*). Theses prenylated isoflavones possess in vitro activity against a number of other *Rhizobium* species. Tahara et al. (*94*) have described the isolation and testing of several flavones, isoflavones (including the novel compound isolupalbigenin, **13**), and chalcone metabolites from the yellow lupin and demonstrated that many of them possess antifungal activity against *Cladosporium herbarum*. Indeed, many novel isoflavones were described for the first time from lupins and reportedly possess antifungal activity (*89, 95–103*).

These studies suggest not only that isoflavones have potential benefits for both human and plant health but also that these traits are subject to manipulation through both farm management and breeding strategies.

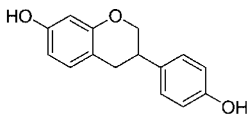
SAPONINS

Saponins are secondary metabolites of mixed biosynthesis. They consist of a triterpene or steroid nucleus (the aglycone) with mono- or oligosaccharides attached to this core. Saponins have long been considered undesirable due to toxicity and their haemolytic activity. However, there is enormous structural diversity within this chemical class, and only a few are toxic (*104*). The most common saponins in legumes include the soyasaponins, which are classified into group A, B, and E saponins on the basis of the chemical structure of the aglycone. These have the general structure shown in **Figure 7**.

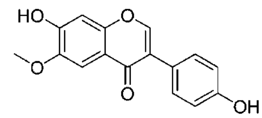
Soyasaponins do not have reported toxicity in monkeys, humans, rats, or chicks, although high levels do impart a bitter taste (*104*). This is not a universal trait of the structure class, and the potential for the use of sweet saponins has been the subject of recent literature (*105, 106*).

Saponins have been reported in many edible legumes, although the detailed structures were not always established. They have been found in lupins (*107, 108*), lentils (*109, 110*), and chickpeas (*104, 110–113*), as well as soy, various beans, and peas (*104*).

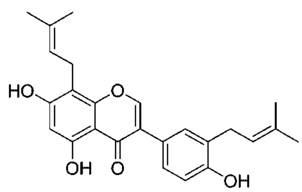
Activities and Bioavailability. The spectrum of biological activity of saponins is as broad as the structure class. The literature suggests that legumous saponins may possess anti-



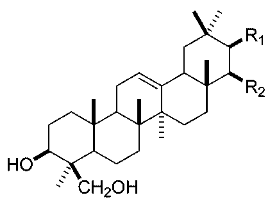
[11] equol



[12] glycitein



[13] isolupalbigenin



Group A saponins R_1=OH, R_2=OH
Group B saponins R_1=H, R_2=OH
Group E saponins R_2=O

Figure 7. Chemical structure of saponins.

cancer activity (*104, 114–119*) and be beneficial for hyperlipidemia (*72, 104, 113*). The adjuvant properties of certain saponins has also been utilized in vaccines for many years (*120*). The best studied are the soyasaponins both in terms of epidemiology and in vitro and in vivo systems.

Epidemiologic studies suggest that saponins may play a role in protection from cancer (*104*), and there are a number of hypothesized modes of action. Mechanistic experiments that give some insight into the potential mode of action of saponins have attracted recent research attention. Metastatic events are critical in cancer proliferation, and there is evidence that glycosylation is an important event in this process (*121–126*). Chang et al. (*115*) have recently demonstrated that soyasaponin I decreases the expression of α-2,3-linked sialic acid on the cell surface, which in turn suppresses the metastatic potential of melanoma cells. The observed anticancer activity may therefore in part be due to this observed sialyltransferase inhibition activity.

Additional mechanistic studies indicate that there is evidence for saponin regulation of the apoptosis pathway enzymes (AKT, Bcl, and ERK1/2), leading to programmed cell death of cancer cells (*116, 127–129*). Research on colon cancer cells suggests that it is the lipophilic saponin cores that may be responsible for the biological activity. The in vitro fermentations carried out by these authors also suggest that colonic microflora hydrolyze soyasaponins to the aglycones, potentially enhancing the activity of the soyasaponins (*119*). This proposed hydrolyzation process is supported by later in vivo and in vitro research that demonstrated that group B soyasaponins were not detected from urine or fecal samples but that the metabolite, soyasapogenol B, was detected in fecal samples. Hu et al. (*118*) showed that uptake by Caco-2 cells was limited, indicating poor intestinal absorption. Studies on saponins from other sources suggest that intestinal uptake is largely by diffusion mechanisms (*130–132*). There is some suggestion that microbial and hepatic modification (esterification with fatty acids) may enhance bioavailability (*133*), but saponins are generally thought to have low bioavailability. There is some evidence that certain materials enhance the absorption of saponins, for example, chitosan and sodium deoxycholate (*131*), and so further research could increase the understanding of additional dietary factors (within or external to the legume) that may enhance uptake. Bioavailability is also influenced by individual metabolism, food processing methods, and interaction with bile acids (*104*), further complicating research in this area.

The hyperlipidemic action of saponins has not been well-studied, and the results can be conflicting (*134*), but some studies suggest that saponins may reduce cholesterol through the formation of an insoluble complex with cholesterol, thus preventing absorption in the intestine. Additionally, some saponins increase the excretion of bile acids—an indirect method of decreasing cholesterol (*135*).

Cooking/Processing Effects. Cooking and processing can have a significant effect on the levels of available saponins in legumes. Interestingly, the results are not necessarily the same for all legumes. Soaking and cooking studies on chickpeas and lentils suggest that 2–5% of saponin content can be lost from chickpeas during cooking, but a much larger 6–14% can be lost from lentils (*110*). The method of cooking has a significant effect on saponin loss, with autoclaving having a large effect (*136*). Some saponins are thermolabile and may interconvert or degrade (e.g., soyasaponin VI forms soyasaponin I with increased cooking times) (*104, 137*). In terms of human health, it is unclear what the biological significance of such interconversions may be.

Agricultural Studies. The role of saponins in the plant is not clear. It is suggested that they play a role in chemical defense. Studies of some lupin saponins show that they possess moderate antifungal activity (*108*), and it is possible that the bitter taste of some saponins, particularly the acetyl derivatives, may act as a deterrent to herbivores (*104*). The saponins of edible legumes are not well-characterized for their natural role in plants. Studies of other plant saponins suggest that many are antimicrobial and may have a role in the protection of plants from microbial infection, a suggestion which is supported by the observation that saponin-deficient strains are often less disease-resistant (*138–148*).

ALKALOIDS

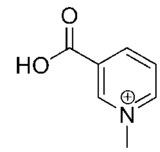
In general, the majority of alkaloids from edible legumes have been reported from lupins. Lupins have a relatively short history of use as a grain crop, and it is only recently (the past 20–30 years) that cultivars have been developed with a reduced alkaloid content (*149*). These cultivars are often referred to as "sweet" lupins since the alkaloids often impart a bitter taste. This is not to say that lupins are the only edible legumes from which alkaloids have been reported. The alkaloid trigonelline (**14**) has been reported from peas (*150*), and it is possible that targeted studies of chickpeas and lentils would also reveal low levels of alkaloids as well.

In particular, it would be interesting to examine some of the less-cultivated landrace varieties of chickpeas and lentils to investigate alkaloid chemistry. Over centuries of cultivated use, alkaloids may well have been bred out of the now-accepted varieties due to toxicity, as well as to enhance palatability. However, such compounds may have biological activity of value in certain circumstances. For example, although toxic to some individuals, hydroxypyrimidine glucoside alkaloids, which are the antimalarial principals of fava beans, have beneficial properties for human health (*151*). Lupins have produced a large range of interesting alkaloid chemicals, and both edible and related lupin species continue to be the subject of literature reporting novel chemistry (*152–158*).

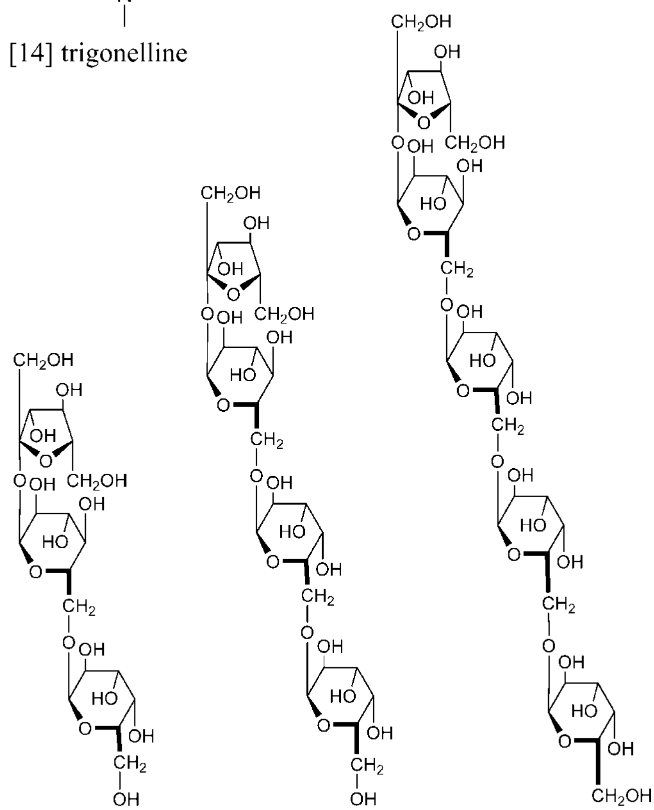
Activities and Bioavailability. As a broad chemical class, alkaloids demonstrate a diversity of biological activity. One of the most intriguing recent reports discusses the enhancement of insulin secretion by lupin quinolizidine alkaloids (*159*). The authors note that this increased secretion only occurs in the presence of relatively high glucose levels and so may be of relevance for managing type II diabetes.

Cooking/Processing Effects. Although there may be trace amounts of alkaloids present in legumes cultivated for human consumption, preparation often removes these chemicals. The alkaloid concentration may be enhanced in the seed (*158*); however, in general, soaking removes a significant proportion of alkaloids from the lupin seed (*160*).

Agricultural Studies. Although the alkaloid content of the legume seeds may be undesirable for human or animal consumption, there is evidence that the alkaloid content is protective for the plant. For instance, it has been noted that "sweet" lupins are susceptible to a large number of insect herbivores to which the wild-type plants are resistant (*161*). Alkaloids are not the only bitter principals that may be responsible for such activity. As has already been discussed, saponins may affect both palatability and disease resistance.



[14] trigonelline



raffinose [15]   stachyose [16]   verbascose [17]

Figure 8. Raffinose family of oligosaccharides.

BIOACTIVE CARBOHYDRATES

Activities and Bioavailability. For many years, the focus on legume sugars and oligosaccharides has centered on the minimization of the raffinose sugars. The link between the raffinose sugars and flatulence has long been understood (*162, 163*) and can be an important impediment to the increased consumption of legumes (*164*). Flatulence and related disorders, including bloating and diarrhea, are due to a lack of α-galactosidases in the upper gut. The oligosaccharides then enter the lower gut where they are metabolized by the bacteria, resulting in an accumulation of carbon dioxide and hydrogen (*165*). The raffinose sugars include raffinose (**15**), stachyose (**16**), and verbascose (**17**) (**Figure 8**). There are appreciable levels of these oligosaccharides in chickpeas, lentils, lupins, and field peas (1–12% of dry weight) with considerable variation between the different pulses (*166*). Studies on the quantities of these sugars in chickpeas show a distinct difference between desi and kabuli types. On average, the desi-type chickpeas are 16% higher in the three oligosaccharides, but with very low levels of verbascose found in either. Interestingly, the kabuli type also has 47% more sucrose than the desi chickpeas (*165*), a likely indication that these are factors subject to breeding (since the kabuli type has been bred for thousands of years, primarily for human consumption).

Oligosaccharides have also been demonstrated to be of potential value for immune health (*167–170*). Evidence for the mode of action is scant, but there are tantalizing hints that such immune modulation may occur through the enhancement of the innate immune system (in particular, through the Tol-like receptors) (*171*). Evidence for immunomodulating oligosaccharides or sugar components for gut health is currently focused more on milk sugars (*172–176*) than those from plants. However, it has been noted that the oligofructose metabolites from plants may play an important role in these functions, and additional research in this area is warranted (*177, 178*).

Phytic acid, or inositol hexaphosphate, has also been identified as an antinutritional, this compound acting through its ability to inhibit mineral uptake. The uptake of zinc, calcium, and iron has been studied, and though the findings are mixed, there is evidence that zinc uptake, in particular, may be inhibited by phytic acid (*179–186*).

Pulse grains are a dietary source of minerals, although their bioavailability is considered lower because of the concentration of phytates (*187*). Phytic acid (myo-inositol-(1,2,3,4,5,6) hexakisphosphate; IP6) is the major source of phosphorous in pulses (*188*), although the concentration is less than 2%; for chickpeas, phytic acid constituents make up 52% of the total available phosphorous (*189*).

Phytic acid has the ability to chelate multivalent ions, those of alkaline metals being soluble in water, while divalent metals such as zinc, calcium, and iron salts are insoluble. This limits the bioavailability of minerals (*190*).

During food processing and digestion, phytase dephosphorylates phytic acid [IP6] to *myo*-inositol pentaphosphate [IP5], *myo*-inositol tetraphosphate [IP4], *myo*-inositol triphosphate [IP3], *myo*-inositol diphosphate [IP2], and *myo*-inositol monophosphate [IP1]; however, only IP5 and IP6 have a negative effect on the bioavailabity of divalent metal ions (*191*).

Of the various forms of phytates, IP6 is present in the greatest quantity and the most stable during cooking, followed by IP5, IP4, and IP3 (**Table 2**).

The ability to chelate with metal ions has associated IP6 with antinutritional properties, although the presence of IP5 and -6 in virtually all mammalian cells (*192*) contradicts this view (*193*), and reports indicate that there may be some protective effects such as decreasing the risk of iron-mediated colon cancer

Table 2. Inositol Phosphate Content of Raw and Cooked Pulses[a]

		IP3 %	IP4 %	IP5 %	IP6 %
chickpea (K)[b]	raw	nd	nd	0.10	0.40
	cooked	nd	0.03	0.12	0.34
chickpea (K)[c]	raw	0.03	nd	0.03	0.38
chickpea (D)[c]	raw	0.03	nd	0.03	0.38
field pea	raw	0.00	0.01	0.08	0.43
	cooked	0.01	0.02	0.10	0.33
lentil	raw	0.02	0.01	0.08	0.55
	cooked	0.05	0.05	0.21	0.47
lentil[c]	raw	0.02	0.02	0.05	0.24

[a] Expressed as a percentage. [b] Reported values multiplied by 420, 500, 580, or 660 to convert from millimoles to milligrams per kilogram for IP3, IP4, IP5, or IP6, respectively (*230*). [c] Adapted from ref *231*; K, Kabuli; D, Desi; nd, not detected.

and lowering serum cholesterol and triglycerides in experimental animals (*194*). Phytic acid has also been shown to have a positive effect due to its antioxidant (*195*) and anticarcinogenic effects (*190, 196*).

IP6 was demonstrated to be effective in controlling cancer in experimental mammary tumours (*197*) and in human prostate carcinoma cells (*198*). Using rats, Shamsuddin demonstrated that IP6 is quickly absorbed through the stomach and upper intestine and is distributed as inositol and IP1, and in in vitro studies, IP6 is taken up by malignant cells undergoing invariable dephosphorylation to IP1–5 and inositol (*199*).

Recently, focus has turned to the potentially beneficial effects of some of these antinutritionals. Phytic acid, in particular, has been the focus of research into its anticancer and hypocholesterolaemic action (*135, 200–205*). Phytic acid has been studied in relation to prostate cancer (*201, 206, 207*), breast cancer (*204*), colon cancer (*208–211*), and leukaemia (*212–214*). The mechanism of the anticancer activity is still not well-understood, though cell arrest at the G1 phase is indicated, potentially acting through the cyclin-dependent kinase pathway (*206, 207*). There is also some indication that phytic acid affects the immune response and that immunomodulating activity may be, in part, responsible for anticancer activity through the activation of natural killer cells (*210*). Regardless of the precise mode of action, there is sufficient excitement around the anticancer activity of phytic acid that some researchers are calling for extensive clinical trials of the phytochemical (*200, 205*).

Cooking/Processing Effects. As mentioned earlier, the removal of oligosaccharides, in particular, of the raffinose family, and undesirable sugars such as phytic acid has been studied for some time. Many of the undesirable oligosaccharides can be dramatically reduced by soaking or cooking (*166, 215–218*). Treatment with α-galactosidases (*219*), germination (*220–223*), and even irradiation (*224*) have also been found to be effective methods to reduce these constituents.

Agricultural Studies. The biological functions of these sugars are not certain, but it seems likely that phytic acid acts as a store of phosphorous within the seed (*225*). In addition, there is evidence that some of the raffinose sugars are protective against cell damage under dehydration conditions (*226, 227*). Genetic studies suggest that these metabolite levels are subject to manipulation (*225, 228*).

Conclusion. The overall benefits of pulses have historically been associated with their macronutrient composition, such as protein and starch, although there is now sufficient evidence to suggest that non-nutritional bioactive compounds play a significant role. Despite a large volume of literature, there is still the need for additional work around the substantiation of the health benefits of different pulse metabolites and the potential synergistic effects between the different classes of bioactives. There is good evidence that levels of these bioactives in the plant are subject to manipulation. There appears to be significant natural variation both between and within the different species of pulses and the opportunity to enhance the concentrations through plant breeding or the application of agronomic practices.

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