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Bioavailability of minerals in legumes

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The mineral content of legumes is generally high, but the bioavailability is poor due to the presence of phytate, which is a main inhibitor of Fe and Zn absorption. Some legumes also contain considerable amounts of Fe-binding polyphenols inhibiting Fe absorption. Furthermore, soya protein *per se* has an inhibiting effect on Fe absorption. Efficient removal of phytate, and probably also polyphenols, can be obtained by enzymatic degradation during food processing, either by increasing the activity of the naturally occurring plant phytases and polyphenol degrading enzymes, or by addition of enzyme preparations. Biological food processing techniques that increase the activity of the native enzymes are soaking, germination, hydrothermal treatment and fermentation. Food processing can be optimized towards highest phytate degradation provided that the optimal conditions for phytase activity in the plant is known. In contrast to cereals, some legumes have highest phytate degradation at neutral or alkaline pH. Addition of microbial enzyme preparations seems to be the most efficient for complete degradation during processing. Fe and Zn absorption have been shown to be low from legume-based diets. It has also been demonstrated that nutritional Fe deficiency reaches its greatest prevalence in populations subsisting on cereal- and legume-based diets. However, in a balanced diet containing animal protein a high intake of legumes is not considered a risk in terms of mineral supply. Furthermore, once phytate, and in certain legumes polyphenols, is degraded, legumes would become good sources of Fe and Zn as the content of these minerals is high.

Pulses: Minerals: Bioavailability: Phytate: Polyphenols: Oxalate

Legumes are rich sources of food proteins from plants and have provided a protein source for man since the earliest civilizations. Legumes are often advocated in Western diets because of their beneficial nutritional effects and because they are a low-cost source of protein. Pulses represent dry grain legumes for human consumption. The content of Fe and other minerals is generally high in legumes (Table 1). However, the legumes also contain antinutritional factors, such as proteinase inhibitors, lectin, raffinose oligosaccharides, saponins, polyphenols and phytate. Antinutritional factors lower the nutritional value of a food by lowering the digestibility or bioavailability of nutrients. Phytate, and some of the degradation products of phytate, are well-known inhibitors of absorption of essential dietary minerals, in particular non-haem iron and Zn. Certain Fe-binding polyphenols are potent inhibitors of non-haem iron absorption.

The negative influence on Fe absorption is nutritionally the most important, especially in industrial products such as infant formulas, but more importantly in many developing countries where diet is based on cereal and legume products. Deficiency of Fe, and perhaps Zn, is highly prevalent in developing countries, but also in vulnerable groups with high requirements in industrialized countries, such as women of fertile age, infants and adolescents. The increased number of vegetarians among young people might lead to increased prevalence of Fe deficiency, because the mineral availability may be crucial in a vegetarian diet (McEndree *et al.* 1983; Helman & Darnton-Hill, 1987; Reddy & Sanders, 1990). In developing countries Fe deficiency, due to poor bioavailability, retards normal brain development in infants and affects the success of a pregnancy by increasing premature deliveries, as well as morbidity of mother and child at or around childbirth. Zn deficiency prevents normal child growth and greatly weakens the immune system, leading to more infections. Nutritional Fe deficiency reaches its greatest prevalence and severity in populations subsisting predominantly on cereal and legume diets (International Nutritional Anemia Consultative Group, 1982).

Phytate

Phytate (inositol hexaphosphate) constitutes 1–3 % of cereal grains, legume seeds and nuts, and also occurs in low concentrations in roots, tubers and vegetables. In particular, wholegrain cereals and legumes have a high

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Table 1. Minerals (iron, zinc, calcium and magnesium; per 100 g of dry seed) in some pulses and soyabeans (from Fachmann *et al.* 2000)

	Fe (mg)	Zn (mg)	Ca (mg)	Mg (mg)
Phaseolus vulgaris	7·0	3·0	197	250
Peas (*Pisum sativum*)	7·36	3·01	96	132
Chickpeas	6·96	3·54	124	155
Lentils	7·50	3·73	71	129
Soyabeans	6·64	4·18	201	220

content of phytate but also of the minerals Zn, Fe and Mg. The phytate content of some legumes is shown in Table 2 and the mineral content in Table 1. In legume seeds, phytate is located in the protein bodies in the endosperm. Phytate occurs as a mineral complex, which is insoluble at the physiological pH of the intestine. It is considered antinutritional, causing reduced uptake in the human intestine of essential dietary minerals such as Fe, Zn and Ca. A dose-dependent inhibition of Fe, Zn and Ca absorption by phytate has been demonstrated in humans (Hallberg *et al.* 1989; Brune *et al.* 1992; Hurrell *et al.* 1992; Fredlund *et al.* 2002). Inositol pentaphosphate has also been identified as an inhibitor of Fe and Zn absorption (Lönnerdal *et al.* 1989; Sandström & Sandberg, 1992; Sandberg *et al.* 1999). Furthermore, it was found recently that inositol tri- and tetraphosphate contribute to the negative effect on Fe absorption of processed food containing a mixture of inositol phosphates (Sandberg *et al.* 1999), probably by interactions with the higher phosphorylated inositol phosphates.

Polyphenols

Certain polyphenols are able to complex-bind Fe, which make the complex-bound Fe unavailable for absorption (Brune *et al.* 1989; Hurrell *et al.* 1999). According to Brune *et al.* (1989) the amount of Fe-binding phenolic galloyl groups in foods roughly corresponds to the degree of inhibition of Fe absorption. Hurrell *et al.* (1999), however, concluded that all major types of food polyphenols can strongly inhibit dietary non-haem iron absorption.

Legumes contain varying amounts of polyphenols and generally the amounts are considered higher in the coloured seeds (reviewed by Salunkhe *et al.* 1982). Beans of the species *Phaseolus vulgaris* were found to contain high amounts of polyphenols (Paredes-Lopez & Harry, 1989), whereas the content of polyphenols in peas (*Pisum sativum*) is very low.

Table 2. Content of phytate-phosphorus in legumes (from Reddy *et al.* 1989)

Legumes	Phytate-P (g/100 g)
Soyabeans	0·28–0·63
Red kidney beans	0·34–0·58
Peas	0·06–0·33
Lentils	0·08–0·30

Oxalic acid

Oxalate salts are poorly soluble at intestinal pH and oxalic acid is known to decrease Ca absorption in monogastric animals (Allen, 1982). The effect of oxalate on Ca absorption in humans is less clear. Ca in spinach with a high oxalate content is very poorly absorbed (Heaney *et al.* 1988) while kale, a low-oxalate vegetable, exhibits excellent Ca absorbability (Heaney & Weaver, 1990). However, studies with calcium oxalate suggest that other factors in spinach contribute to the low Ca absorption (Heaney & Weaver, 1989). A certain amount of oxalic acid also occurs in phaseolus beans.

Enzymatic degradation of phytate in legumes during food processing

Degradation of phytate can occur both during food processing and in the gastrointestinal tract. This degradation is of nutritional importance because it has been demonstrated that such controlled degradation improves the uptake of essential dietary minerals, i.e. Fe and Zn (Sandberg & Svanberg, 1991; Brune *et al.* 1992; Hurrell *et al.* 1992). Major efforts are therefore made to reduce the amount of phytate in foods by means of phytate-degrading enzymes, phytases, present naturally in the plant foods or present in yeasts or other micro-organisms used in food processing. These enzymes successively remove, one after the other, the six phosphorus groups attached to the inositol ring. Biotechnologically produced microbial phytase preparations are now commercially available and used for feed preparations. In the future, their use in food processing could be feasible. In order to substantially increase Fe absorption, phytate degradation has to be virtually complete. Recent findings suggest that the inositol penta-, tetra- and triphosphates must also be degraded in order to improve Fe absorption (Sandberg *et al.* 1999).

However, ascorbic acid is a potent enhancer of Fe absorption, which can counteract the inhibitory effect of phytate (Hallberg *et al.* 1989; Siegenberg *et al.* 1991). Increasing the ascorbic acid in phytate-containing meals is thus another means of improving Fe absorption. Certain other organic acids formed during fermentation may also improve Fe absorption. Heat treatment of plant foods, on the other hand, often reduces the bioavailability due to inactivation of the enzyme phytase and destruction of ascorbic acid (reviewed by Sandberg, 1996).

Biological food-processing techniques that increase the activity of native enzymes of cereals and legumes are: soaking, germination, hydrothermal processing and fermentation. During germination, phytase enzymes are synthesized or activated. Lactic fermentation leads to lowering of pH as a consequence of bacterial production of organic acids, mainly lactic acid, which is favourable for cereal phytase activity (Sandberg, 2002). The micro-organisms (e.g. fungi) of the starter culture used in fermentation, in some cases, exert phytase activity. However, in contrast to fungi and yeast, *Lactobacillus* sp. was not found to produce phytase (Fredrikson *et al.* 2002*b*).

To optimize the food process to increase mineral availability by phytate degradation, it is essential to know the optimal conditions for the phytases, which are responsible for phytate degradation in the food process. There are differences in optimal conditions for phytate degradation between plant species. Most cereal phytases have pH optima between 4·5 and 5·6, but pH optima of some legumes are neutral or alkaline (Loewus *et al.* 1990). Scott (1991) demonstrated an alkaline phytase extracted from different varieties of *Phaseolus vulgaris*. Gustafsson & Sandberg (1995) found that phytate degradation occurs in brown beans at pH 4·5 and at pH 8 at 37°C, but the highest phytate degradation was found at pH 7·0 and 55°C. By fermentation of pre-soaked beans, an 88 % reduction of the phytate content was obtained.

In germinated or soaked lentils, faba beans and peas, the highest phytate degradation was found at 45°C and pH 5·0, 4·0 and 7·0, respectively (Honke *et al.* 1999). Fredrikson *et al.* (2001*a*) achieved the most efficient phytate degradation of pea flour at pH 7·5 and 45°C. At these soaking conditions it was possible to obtain an almost complete phytate degradation in 10 h, combined with 66 % reduction of the sum of inositol penta-, tetra- and triphosphates. Favourable conditions for phytate degradation in black beans were found at pH 5·5 and 50°C (Greiner & Konietzny, 1997). However, compared to soaking at the mentioned conditions, a more extensive degradation was obtained by adding exogenous phytase.

Enzymic degradation of polyphenols in legumes during processing

Enzymic degradation of polyphenols during processing should also be a possible strategy for improvement of Fe availability. Moreover, the addition of ascorbic acid was found to prevent the dose-dependent inhibitory effect of polyphenols on non-haem iron absorption (Siegenberg *et al.* 1991).

At least for high-tannin cereals, it has been shown that treatment with polyphenol oxidase had a reducing effect on the phenolic content (Matuschek *et al.* 2001). Addition of 1500 U/g resulted in a 60 % reduction of phenolic compounds and a significant improvement of Fe availability, estimated *in vitro*. A fungal tannase was used to decompose phenolic compounds in brown beans, but the influence on Fe availability was not determined (Gustafsson & Sandberg, 1995). Germination and fermentation of lentils were found to modify the phenolic composition. Germination reduced the amount of phenolic compounds, while natural fermentation was found to increase certain phenolic monomers (catechin) (Bartolomé *et al.* 1997).

Iron and zinc absorption from legume-based diets

Fe absorption from soyabeans and soya protein products studied in single meals, using extrinsic labelling of meals with radioactive isotopes or studied by a stable isotope technique, was found to be low (Cook *et al.* 1981; Morck *et al.* 1981; Hallberg & Rossander, 1982; Hurrell *et al.* 1992; Davidsson *et al.* 1994). Fe absorption from single meals based on black beans, lentils, mung beans and split beans was found to be very low, ranging from 0·8 % to 1·9 % (Lynch *et al.* 1984).

Studies of meals based on white beans and soyabeans indicate that the effect of phytate on Zn absorption is less pronounced in soyabeans than in cereals (Sandström & Cederblad, 1980; Sandström *et al.* 1989) and that legume and animal protein are comparable, with the same Zn content. Low Zn absorption from a soya-protein-based infant formula has, however, been found (Sandström *et al.* 1983).

The enzymatic degradation of phytate, by addition of a microbial phytase preparation, in soya infant formula was found to improve Fe absorption significantly, provided that the removal of phytate was virtually complete (Hurrell *et al.* 1992). However, in the same study it was found that even after removal of phytate, soya protein itself is still relatively inhibitory to Fe absorption, probably due to the presence of Fe-binding peptides. Davidsson *et al.* (1994) demonstrated that the Fe bioavailability can be increased by either removal of phytate or increasing the ascorbic acid content in soya-based infant formulas. It has also been demonstrated that removal of phytate from soya formulas significantly improves Zn absorption (Lönnerdal *et al.* 1988).

The New Technologies for Improved Nutritional and Functional Value of Pea Protein project

The general objective of the EU-funded project, NUTRIPEA (FAIR CT 95–0193), was to use new technologies to develop improved pea protein products, which are devoid of antiphysiological and antinutritional factors. The nutritional and functional properties of pea proteins suggest a high potential for use in food products. Therefore, the purpose of the project was to design and develop a technical process: (1) to prepare improved pea protein products under pilot plant and factory conditions; (2) to evaluate the functional and sensory properties of improved pea products added to a variety of foods for human consumption; (3) to screen *in vitro* and in animal models the nutritional properties and antigenicity of the protein products; (4) to develop an infant formula based on the improved pea protein products; and (5) to evaluate the formula for antigenicity and protein quality in animals and Fe absorption in humans. The results have been published recently (Sandberg, 2000).

The use of pea protein isolate could be an alternative to soya isolate. Soya formulas have been used for a long period and the nutritional status of infants fed soya formula has been well documented and found to be similar to that of infants fed cow's milk formulas. However, the bioavailability of nutrients, especially minerals, has been reported to be lower than that of milk-based formulas. The availability of Fe and Zn in a dephytinized infant formula based on pea protein was evaluated (Fredrikson *et al.* 2001*b*, 2002*a*). Soluble amounts of Fe and Zn were collected during simulated *in vitro* digestion performed in a computer-controlled dynamic gastrointestinal model. The results showed that the complete dephytinization of pea protein, by addition of a microbial phytase preparation during processing, increased the amount of Fe and Zn potentially available for absorption, by 50 % and 100 %, respectively. Also, an improved uptake of Fe was demonstrated *in vitro* in Caco 2 cells (Fredrikson *et al.* 2002*a*). The improvement of Fe availability was confirmed in a human study, the Fe absorption increasing by more than 50 % after phytate removal or addition of ascorbic acid to the pea protein infant formula (Davidsson *et al.* 2001). The data in the human study also suggest that fractional Fe absorption is higher from the dephytinized pea protein formula compared to dephytinized soya protein formulas. Pea protein may therefore be a nutritional beneficial alternative to soya protein in infant formulas, but further evaluation is needed.

Conclusions

The absorption of minerals depends on the total composition of the meal. In a balanced diet containing animal protein, a high intake of legumes does not imply a risk of inadequate mineral supply. Strictly vegetarian diets based on unrefined cereals and possibly also some legume-based diets will result in low absorption of Fe and Zn. However, the utilization of Fe and Zn, and probably other minerals, can be improved by food processes such as fermentation with phytase-producing micro-organisms; soaking and germination, which degrade phytate; or addition of phytase and, in some legumes, polyphenol-degrading enzymes. In the modern food industry, the phytate content of soya-based infant formulas is of concern; major efforts are therefore being made to remove phytate. Once phytate is degraded, legumes would become good sources of Fe and Zn as the content of these minerals is high.

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