Improving the bioavailability of nutrients in plant foods at the household level

Rosalind S. Gibson*, Leah Perlas† and Christine Hotz‡

Department of Human Nutrition, University of Otago, Dunedin, New Zealand

Plant foods are the major staples of diets in developing countries, in which the consumption of animal-source foods is often low because of economic and/or religious concerns. However, such plant-based diets are often associated with micronutrient deficits, exacerbated in part by poor micronutrient bioavailability. Diet-related factors in plant foods that affect bioavailability include: the chemical form of the nutrient in food and/or nature of the food matrix; interactions between nutrients and other organic components (e.g. phytate, polyphenols, dietary fibre, oxalic acid, protein, fat, ascobic acid); pretreatment of food as a result of processing and/or preparation practices. Consequently, household strategies that reduce the content or counteract the inhibiting effects of these factors on micronutrient bioavailability are urgently needed in developing-country settings. Examples of such strategies include: germination, microbial fermentation or soaking to reduce the phytate and polyphenol content of unrefined cereal porridges used for young child feeding; addition of ascorbic acid-containing fruits to enhance non-haem-Fe absorption; heating to destroy heat-labile anti-nutritional factors (e.g. goitrogens, thiaminases) or disrupt carotenoid–protein complexes. Such strategies have been employed in both experimental isotope-absorption and community-based studies. Increases in Fe, Zn and Ca absorption have been reported in adults fed dephytinized cereals compared with cereals containing their native phytate. In community-based studies in rural Malawi improvements in dietary quality and arm-muscle area and reductions in the incidence of anaemia and common infections in young children have been observed.

Bioavailability: Plant foods: Household: Micronutrients: Phytate
The absorption and/or utilization of the trace elements Fe, Se and Zn, and of the vitamins niacin, provitamin A carotenoids and folate are most affected by their chemical form. Of these micronutrients, the bioavailability of the two forms of Fe in food (haem- and non-haem-Fe; Hallberg, 1981), certain isomeric forms of carotenoids (Yeum & Russell, 2002) and folate polyglutamates relative to monoglutamates (McNulty & Pentieva, 2004) have been reviewed in detail elsewhere.

The food matrix probably has the greatest effect on the absorption of provitamin A carotenoids and folate, both of which can be entrapped in the insoluble plant matrix, thus reducing their bioavailability. For example, β-carotene in raw carrots or lycopene in fresh tomato juice are poorly absorbed compared with pure β-carotene dissolved in oil (Yeum & Russell, 2002), whereas the bioavailability of folate from chopped spinach (Spinacia oleracea) is higher than that from whole spinach leaves (Castenmiller et al. 2000).

### Chemical form of the nutrient and nature of the dietary matrix

In general, diet-related factors have a greater influence on the bioavailability of the micronutrients in plant foods, particularly Ca, Fe and Zn, than on the macronutrients. The absorption of Ca, Fe and Zn is particularly affected. The net effect on the nutrient bioavailability depends on the balance between factors that either inhibit or enhance nutrient absorption and/or utilization in the whole diet (Sandström, 2001). Increasingly, the influence of both synthetic micronutrient fortificants and intrinsic micronutrients on micronutrient bioavailability must be considered.

### Interactions between nutrients themselves and with other organic components in the plant food

Direct competitive interactions between two (or more) inorganic nutrients with similar physico-chemical properties that share the same absorptive pathways are unlikely in plant foods because the intrinsic micronutrient levels are low. Even when plant staples serve as vehicles for fortification the risk of such interactions is small because of the presence of dietary ligands in food (Sandström et al. 1985).

In contrast, there are several organic components in plant foods that may form insoluble or soluble complexes with certain micronutrients in the acid pH of the stomach and gastrointestinal tract, thus inhibiting or facilitating their absorption. Re-absorption of endogenously-excreted Ca, Zn and Cu may also be affected (Sandström, 2001; Manary et al. 2002a). Examples of these non-competitive interactions are summarized in Tables 1 and 2; both inhibiting and enhancing factors are listed.
Table 2. Effects of non-competitive interactions involving organic substances on nutrient bioavailability in plant foods: enhancing factors

<table>
<thead>
<tr>
<th>Dietary component</th>
<th>Food sources</th>
<th>Main technical influences</th>
<th>Nutritional consequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Organic acids (citric, lactic, acetic, butyric, propionic and formic acids)</td>
<td>Fermented milk products (e.g. yoghurts), vegetables, sauerkraut, soya sauces, fermented cereals (e.g. Tobwa)</td>
<td>May form soluble ligands with some trace minerals in the gastrointestinal tract</td>
<td>Enhance absorption of Zn and possibly Fe (Sandström, 1997; Teucher et al. 2004)</td>
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<tr>
<td>Ascorbic acid</td>
<td>Citrus fruits and juices Other fruits: guavas, mango, papayas, kiwi, strawberries Vegetables: tomato, asparagus, Brussels sprouts, spinach etc.</td>
<td>Reduces Fe$^{3+}$ to more soluble Fe$^{2+}$; forms Fe–ascorbate chelate May increase stability of folate during food processing and digestion</td>
<td>Enhances non-haem-Fe absorption (Teucher et al. 2004) Counteracts inhibitory effect of phytate May enhance folate bioavailability (McNulty &amp; Pentieva, 2004) May enhance or inhibit Se absorption, depending on the chemical form (Mutanen &amp; Mykkänen, 1985; Levander, 1987) Ascorbic acid may also enhance Cr absorption (Offenbacher, 1994)</td>
</tr>
<tr>
<td>Protein</td>
<td>Amount and type (e.g. animal protein) form soluble ligands with Zn, Fe and Cu</td>
<td></td>
<td>Enhance absorption of Zn, Fe and Cu (Bjorn-Rasmussen &amp; Halberg, 1979; Turnlund et al. 1983; Lönnertal, 2000) Increase urinary Cu excretion (Heaney, 2000)</td>
</tr>
<tr>
<td>Fat</td>
<td>Oil seeds, nuts Products of fat digestion + bile salts solubilize fat-soluble vitamins and carotenoids in intestinal milieu</td>
<td></td>
<td>Enhance absorption of fat-soluble vitamins and provitamin A carotenoids (Yeum &amp; Russell, 2002)</td>
</tr>
</tbody>
</table>

Guava, Psidium guajava L.; mango, Mangifera indica L.; papaya, Carica papaya; kiwi, Actinidia deliciosa; strawberry, Fragaria X ananassa; asparagus, Asparagus officinalis; spinach, Spinacia oleracea.

Pretreatment of food in the household as a result of processing and/or preparation practices

The adverse effects of some of the organic components in plant foods on nutrient bioavailability can be reduced by household food processing and preparation practices; these practices are summarized in Table 3 and will be discussed.

Thermal processing. This treatment generally enhances the digestibility of proteins and carbohydrates, although if Maillard browning occurs in baked foods protein quality and digestibility may be reduced (Table 3). Thermal processing may also improve the bioavailability of certain vitamins and I, in some cases because of the destruction of heat-labile anti-nutritional factors (Erdman & Pheros-Schneier, 1994). For example, thiaminases in Brussels sprouts and red cabbage, which catalyse the cleavage of thiamin, are destroyed by cooking (Hilker & Somogyi, 1982). Cooking also destroys the goitrogens present in cabbage, Brussels sprouts, turnips, sweet potatoes (Ipomoea batatas), millet (Achnatherum hymenoides), cassava (Manihot esculenta Crantz) and beans. Such goitrogens block the absorption or utilization of I and thus its uptake into the thyroid gland (Gaitan, 1990).

Thermal processing can also enhance the bioavailability of vitamins such as thiamin, vitamin B$_6$, niacin and carotenoids by releasing them from entrapment in the plant matrix. For example, greater increases in total serum β-carotene and serum lycopene have been reported after eating cooked carrots and spinach (Rock et al. 1998) and cooked tomatoes (van het Hof et al. 2000) compared with levels when they are consumed raw. This effect is attributed to softening or disruption of plant cell walls and the disruption of carotenoid–protein complexes so that the carotenoids are more available in the intestinal lumen for absorption (Yeum & Russell, 2002).

Reports on the effects of thermal processing on phytate degradation are inconsistent and depend on the plant species, temperature and/or pH. Hurrell et al. (2002) have reported that home thermal processing does not degrade phytate sufficiently to improve Fe absorption from home-prepared pancakes or chapattis. Other investigators (Kataria et al. 1989; Marfo et al. 1990; Khan et al. 1991) have suggested that conventional heat treatments such as boiling may induce moderate losses (i.e. 5–15%) of phytic acid in tubers (Marfo et al. 1990) and some legumes (Kataria et al. 1989) and cereals (Khan et al. 1991). Much higher losses have been reported after boiling white rice (i.e. 70%; Perlas & Gibson, 2002), attributed mainly to leaching of water-soluble sodium, potassium or magnesium phytate into the discarded cooking water.

Germination. This process, also termed malting, leads to an increase in phytase activity in certain cereals (e.g. maize, millet and sorghum (Sorghum bicolor (L.) Moench)), in most legumes and in oil seeds through de novo synthesis and/or activation of intrinsic phytase.
Tropical cereals such as maize and sorghum have a lower endogenous phytase activity than do rye, wheat, triticale (*X Triticosecale* Wittmack), buckwheat (*Fagopyrum esculentum*) or barley (Egli et al. 2002). Phytases (*myo*-inositol hexakisphosphate 3-phosphohydrolase) hydrolyse phytic acid (*myo*-inositol hexaphosphate) as well as the salts, magnesium, calcium or potassium phytate, to yield inorganic orthophosphate and *myo*-inositol via intermediate *myo*-inositol phosphates (pentaphosphates to monophosphates). The rate of phytate hydrolysis varies with the species and variety, as well as the stage of germination, pH, moisture content, temperature (optimal range 45–57°C), solubility of phytate and the presence of certain inhibitors (Cheryan, 1980; Egli et al. 2003). Egli et al. (2002) have observed that during germination rice, millet and mungbean (*Vigna radiata* L.) have the largest reductions in phytate content, ranging from 50% (for mungbeans) to 64%.

Such reductions in the levels of higher inositol phosphates can have a major impact on mineral bioavailability because they form complexes with divalent and trivalent cations (particularly Zn, Fe, Ca and Mg) at the physiological pH conditions of the small intestine, making them unavailable for absorption (Obereis & Harland, 1981; Hurrell, 2003; Egli et al. 2004; Hurrell et al. 2004); the higher inositol phosphates have no effect on Cu absorption (Egli et al. 2004). The hexa- and pentaphosphates may also complex endogenously-secreted minerals such as Zn (Sandström, 1997; Manary et al. 2000) and Ca (Morris & Ellis, 1985), making them unavailable for re-absorption into the body. In contrast, *myo*-inositol phosphates with less than five phosphate groups (i.e. monophosphates to tetraphosphates) do not have a negative effect on Zn absorption (Lörnerdal et al. 1989) and those with less than three phosphate groups do not inhibit non-haem-Fe absorption (Sandberg et al. 1999). There appears to be no adaptation to the inhibitory effect of a high-phytate diet on absorption of Fe (Brune et al. 1989) or exogenous Zn, although endogenous excretion of faecal Zn may be decreased in healthy subjects (Sandström et al. 1993).

Certain tannins and other polyphenols in legumes (e.g. *Vicia faba*) and red sorghum may also be reduced during germination as a result of the formation of polyphenol complexes with proteins and the gradual degradation of oligosaccharides (Camacho et al. 1992). Naturally-occurring polyphenol oxidase extracted from banana (*Musa X paradiasica* L.) or avocado (*Persea americana* Mill.) and subsequently reduced by dialysis has also been used to reduce the polyphenol content of high-tannin sorghum (Matuschek & Svanberg, 2004).

α-Amylase activity is also increased during germination of cereals, particularly sorghum and millet. This enzyme hydrolyses amylose and amylpectin to dextrins and maltose, thus reducing the viscosity of thick cereal porridges (Gibson & Ferguson, 1998). A threefold increase in Fe absorption has been reported in amylase-treated
roller-dried rice cereal compared with the untreated roller-
dried cereal, which is associated with the viscosity changes
induced by α-amylase (Hurrell et al. 2002).

Milling or household pounding. In developing coun-
tries this process is used to remove the bran and/or germ
from cereals such as wheat, sorghum, rice and maize.
These processes also reduce the phytate content if the
phytate is localized in the outer aleurone layer (e.g. rice,
sorghum and wheat) or in the germ (i.e. maize; O’Dell et
al. 1972). Milling can thus enhance mineral bioavail-
ability, although the content of minerals and some vitamins
of the milled cereals are simultaneously reduced. As a
result, in some countries milled cereal flours are enriched
to compensate for the micronutrients lost.

Microbial fermentation. Higher inositol phosphates are
hydrolysed to lower inositol phosphates through the action
of microbial phytase enzymes (Sandberg, 1991). These
micro-organisms may occur naturally on the surface of
cereals and legumes or can be introduced via inoculation
with a starter culture. The extent of the reduction in higher
inositol phosphate levels depends on the type of fer-
tmentation; sometimes ≥90% phytate can be removed by
fermentation of maize, soyabean, sorghum, cassava,
cocoyam (Colocasia esculenta), cowpeas (Vigna unguicu-
latata) and lima beans (Phaseolus limensis; Marfo et al.
1990; Sandberg, 1991; Svanberg et al. 1993). Fermentation
of bread dough with yeast also induces phytate hydrolysis,
although if Ca is added as a fortificant phytase activity in
yeast is inhibited (Hallberg et al. 1991).

Organic acids are also produced during fermentation
and can potentially enhance Fe and Zn absorption via the
formation of soluble ligands (Charlton, 1983; Hazell &
Johnson, 1987; Walter et al. 1998; Porres et al. 2001).
They may also complex some of the minerals bound
to phytate molecules, rendering them more susceptible
to hydrolysis via phytase enzymes (Maenz et al. 1999),
while simultaneously generating a pH that optimizes the activity
of intrinsic phytase from cereal or legume flours (Porres
et al. 2001). In contrast, organic acids may have an inhibi-
tory effect on the activity of the intestinal brush-border
enzyme glutamate carboxypeptidase II, attributed to a
lowering of the pH (McNulty & Pentieva, 2004).

Improvements in protein quality have also been docu-
mented after fermenting blended mixtures of plant-based
complementary foods based on maize and legumes (Nnam,
1999), groundnuts (Apios americana Medic), pumpkin
(Cucurbita Pepo) and millet seeds (Ezeji & Ojimelukwe,
1993) and cereal and soyabean blends (Sanni et al. 1999).
Such improvements may be associated with the destruction
by microbial enzymes of protein inhibitors that interfere
with N digestibility (Nnam, 1999), or from the ability
of starter cultures to synthesize certain amino acids (Odunfa,
1985).

Soaking. The soaking of cereal and most legume flours
in water results in the passive diffusion of water-soluble
sodium, potassium or magnesium phytate, which can be
removed by decanting the water (De Boland et al. 1975;
Chang et al. 1977; Perlas & Gibson, 2002). Nevertheless,
the extent of the removal depends on the species, pH and
length and conditions of soaking. Reductions in the penta-
and hexaphosphates of 47, 57 and 98% respectively have
been reported for mungbean, maize and rice flours after
soaking (Hotz & Gibson, 2001; Perlas & Gibson, 2002;
Temple et al. 2002); however, no reductions are achieved
after soaking whole mungbeans and maize kernels for 6 h
(Perlas & Gibson, 2002; Temple et al. 2002). Reductions
in the content of other anti-nutrients such as glycosides,
alkaloids, oligosaccharides, saponins, polyphenols and
oxalates may also occur (Chang et al. 1977).

Application of household processing and preparation
strategies to enhance nutrient bioavailability of plant
foods in developing countries

There is an urgent need to improve the nutritional quality
of plant-based foods in developing countries, especially
those used for feeding infants and young children. In the
past the emphasis has been on enhancing their protein
quality by blending cereals with legumes (usually in ratios
of 70:30 (w/w) to provide the optimal mixture of essential
amino acids), and problems associated with mineral bio-
availability have often been ignored. This approach is
fortunate because many of these cereal–legume blends
have a very high phytate content and high phytate:Zn and
phytate:Fe molar ratios.

The inhibitory effect of phytate on Zn absorption follows
d a dose-dependent response (Navel et al. 1985) and
the molar ratio for phytate:Zn in the diet is used to esti-
more the proportion of absorbable Zn (Oberleas & Harland,
1981). For Fe, phytic acid begins to lose its inhibitory
effect on Fe absorption when ratios are <1:0:1:0 and it
still inhibits Fe absorption at ratios as low as 0:2:1:0

Both in vitro and in vivo methods have been used to
estimate the bioavailability of Fe, Zn and Ca in plant
foods. Some in vitro methods are based on a two-stage
simulated digestive process of the food or test meal,
followed by determination of the dialysable Fe, Zn or Ca
released. In general, the magnitude of the responses
measured using these methods are not the same as those
observed in human subjects, but some of these methods
have been used to rank foods with respect to the effect of
processing and preparation practices on mineral bioavail-
ability (Latunde-Dada et al. 1998). For example, increases
in dialysable Fe, Zn and Ca have been reported after
processing porridges prepared from legumes such as
chickpea (Cicer arietinum) and black gram (Phaseolus
mungo) flours and/or cereal flours such as maize, sorghum
and rice by fermentation with a starter culture (Svanberg
et al. 1993; Jood & Kapoor, 1997; Sharma & Khetarpaul,
1998) and/or by soaking and germination (Svanberg et al.
2002; Mbithi-Mwikya et al. 2002).

More recently, cultured human intestinal cells (i.e. a
Caco-2 cell in vitro model) have been developed for
studying the characteristics of Fe, and in some cases Zn
and Ca, transport by the intestinal absorptive epithelium
(Han et al. 1994; Wortley et al. 2005). However, currently,
there is no standardized Caco-2 cell method and the mag-
nitude of the effects observed appears to depend on the
procedures used, making inter-laboratory comparisons
difficult. Studies have applied this technique to screen and
rank selected staple-food genotypes for bioavailable Fe (Van Campen & Glahn, 1999), but further development of the Caco-2 cell model is needed before it can be used to determine the bioavailability of Zn and provitamin A carotenoids in plant foods.

To date, in vivo isotope studies to measure the bioavailability of Fe or Zn in plant foods modified using household strategies to enhance Fe or Zn absorption are limited. Studies on adults have demonstrated increases in Fe and Zn absorption when they are fed porridges used for infant feeding that have been prepared from a variety of dephytinized cereals as compared with those containing their native phytate. In a study of Fe absorption (Hurrell et al. 2003) phytic acid was degraded by the addition of exogenous commercial phytase enzyme, whereas in a Zn absorption study (Egli et al. 2004) dephytinization of a wheat–soyabeans blend was achieved by the use of phytase naturally occurring in wholegrain cereals (Egli et al. 2003). In a hospital-based study in Malawi (Manary et al. 2000) the reduction of phytate in a maize–soyabean porridge by using a commercial phytase enzyme has been shown to increase fractional and total Zn absorption and reduce endogenous Zn losses in children recovering from tuberculosis but has no effect on Zn absorption in apparently-well children.

Only a few community-based efficacy trials have assessed the impact of food-based strategies in the household designed to enhance nutrient bioavailability. Early studies focused on improving the bioavailability of non-haem-Fe have been reviewed by Ruel (2001). More recent studies have measured various outcomes, including absorption in vivo using stable isotopes of Fe (Diaz et al. 2003), nutrient adequacy (Gibson et al. 2003; Hotz & Gibson, 2005) and biochemical and/or functional health outcomes (Dewey et al. 1997; Manary et al. 2002b; Yeudall et al. 2002; Garcia et al. 2003; Mamiro et al. 2004).

In a recent study in rural Mexico no improvement in biochemical Fe status was observed among Fe-deficient women receiving 25 mg ascorbic acid from fresh lime juice twice daily on 6 d/week for 8 months compared with those receiving a placebo (Garcia et al. 2003), despite a twofold increase in Fe absorption, based on earlier stable-isotope results (Diaz et al. 2003). Similarly, after withholding coffee for 5 months no positive effect on Fe status was observed among Fe-deficient Guatemalan toddlers, except among those taking Fe supplements (Dewey et al. 1997), attributed to the relatively small amount of coffee ingested. Furthermore, in a large community-based double-blind randomized controlled trial in Tanzania (Mamiro et al. 2004), in which a processed complementary food (based on soaked and germinated finger millet (Eleusine coracana) and kidney beans (Phaseolus vulgaris), with roasted peanuts (Arachis hypogea) and mango (Mangifera indica L.) puree) and an identical unprocessed blend were fed to 6-month-old infants (n 309) for 6 months, no significant differences were found between the two groups at the end of the study in either Fe status, as measured by Hb and zinc protoporphyrin, or growth, perhaps in part because there was only a 34% reduction in the phytate content of the processed complementary food.

These results emphasize that an integrated approach that combines a variety of the strategies discussed earlier, including the addition of even a small amount of animal-source foods, is probably the best strategy to improve the nutrient bioavailability in diets based on plant foods. Two such community-based efficacy trials have been undertaken among weanlings and young children in rural Malawi. Both trials employed a quasi-experimental design with non-equivalent control groups and used a participatory approach to implement a combination of food-based strategies in the household to enhance their awareness, feasibility and acceptability to caregivers in the local community. Details of the strategies and their implementation have been published (Gibson et al. 1998, 2003; Yeudall et al. 2002, 2005; Hotz & Gibson, 2005); their efficacy was evaluated by determining knowledge, trial and adoption of the new practices and comparing dietary quality and the adequacy of the energy and nutrient intakes of the intervention and control groups post-intervention (Gibson et al. 2003; Hotz & Gibson, 2005) and, for the children only, changes in growth and body composition, morbidity and Hb and hair Zn concentrations (Yeudall et al. 2002).

Results of the Malawian studies suggest that a combination of household food-based strategies, comparable with those outlined earlier, can be designed to be feasible and acceptable to caregivers of weanlings and children in subsistence farming settings, although on-going nutrition education and social marketing efforts are required to enhance their adoption and to empower the community to sustain them. Nevertheless, even when such a combination of strategies is used, they are probably not sufficient to overcome the deficits in Ca, Fe and Zn, and possibly other micronutrients that exist in complementary diets in low-resource settings. In such cases additional strategies to enhance the micronutrient adequacy of these complementary diets are urgently required. Possible strategies include fortifying cereal-based dephytinized complementary foods with a fortificant containing balanced and physiological levels of multi-micronutrients. In the long term, biofortification of staple cereals involving strategies to enhance both micronutrient density and bioavailability may become a feasible option for improving the micronutrient status of the entire household in poor-resource settings.

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