NUTRITIONAL ASPECTS OF TRADITIONAL GRAINS

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INTRODUCTION

Malnutrition and deficiency of micronutrients are highly prevalent and even increasing in many low-income countries (WHO 2001). It is estimated that more than 500 million people (mainly children) are underweight and even more people are affected by micronutrient deficiencies, and three focal nutrients are regarded as the major ones, i.e. vitamin A, iron and iodine. Of these, iron deficiency is regarded as the most common nutritional disorder in the world and affects over one billion people (WHO 2001), particularly the vulnerable groups in tropical and sub-tropical zones. Factors of immediate and direct influence on these nutritional disorders are inadequate food consumption and diseases. In addition, the bioavailability of many nutrients in plant based diets is usually low, and this will significantly contribute to the nutritional inadequacy. Cereals, legumes, tubers and oil seeds provide the major bulk of dietary proteins, calories, vitamins, and minerals to the population in developing countries. Biofortification and biological processing techniques are means to enhance the nutritional quality of such foods. On household level, one traditional technology that is widely practised is the fermentation technique. This paper will thus focus on traditional processing techniques with the aim to improve the bioavailability of dietary iron in cereals.

DIETARY IRON INTAKES

Iron is present in many food items. The iron content of different African foods (FAO 1968) is relatively high, and the cereals (maize, sorghum and millets) are regarded as good sources of non-haem iron. These cereals are supposed to supply on average more than 60% of the dietary iron in Africa. Data on dietary intakes are, however, scanty and present wide variations from one survey to another. The data suggest intakes of 10 mg/day and these data are consistent with an analysis of food balance sheets (FAO 1980), which show that total iron per capita in the African diet varies from 14 to 21 mg/day. These amounts are generally considered to be high enough to satisfy the daily iron requirements. However, a large proportion of this iron is poorly absorbed resulting in inadequate iron nutrition of the individual.

ANTI-NUTRITIONAL FACTORS

Most cereal-based diets have poor bioavailability of iron as a result of the presence of anti-nutritional factors such as phytate and in some varieties significant amounts of phenolic compounds, including tannins.

Phytate

Phytate (*myo*-inositol hexaphosphate, IP_6) is an abundant plant constituent of all cereals, nuts, legumes and oil seeds (Erdman 1979). It is one of the most important factors determining iron bioavailability in plant foods (Reddy et al., 2000). It is strongly negatively charged at neutral pH values, and it has a high affinity for cat ions such as iron, zinc, calcium and magnesium. It is well known that the molecule of phytate can be dephosphorylated by means of enzymes or as a result of high-temperature processing to yield a large number of positional isomers: *myo*-inositol bis-, tris-, tetra-, penta- and hexaphosphates (IP_2-IP_6) (Sandberg and Ahderinne 1986). Thus, during food processing and digestion in the human gut, it is likely that lower inositol phosphates are formed (Sandberg and Andersson 1988; Svanberg and Sandberg

1988). Normally, the divalent cat ions form insoluble penta- and hexasubstituted salts (Sandberg et al., 1999).

Polyphenols (tannins)

Phenolic compounds are almost ubiquitous in plants, and high amounts are found in tea, coffee, wine and herbs, as well as in coloured varieties of cereals and legumes. Polyphenols were discovered to be inhibitors of iron absorption in the 1970s when the inhibitory effect of tea was explained by its high content of polyphenols (Disler et al., 1975). They comprise a diverse group of compounds, but it is mainly phenols containing catechol (*ortho*-dihydroxyl) and/or galloyl (trihydroxyl) groups that form insoluble complexes with iron (Brune et al., 1991; Slabbert 1992). Brune et al. (1989) showed that several different types of phenolic compounds, both low- and high-molecular weight phenols, inhibit iron absorption. Hurrell et al. (1999) furthermore suggested that all major types of phenolic compounds inhibit iron absorption, *i.e.* phenolic acids, monomeric flavonoids and complex polymerization products. Several independent studies have shown that the inhibitory effect of phenolic compounds is dose dependent (Brune et al. 1989; Hurrell et al. 1999; Layrisse et al. 2000).

AVAILABILITY OF IRON

Effect of phytate degradation

The inhibition of non-haem iron absorption by phytates is dose dependent and even low levels (less than 90% of whole wheat flour phytate content) exhibit a strong inhibition (Brune et al., 1992). These results show a strong semi-logarithmic relationship (r = 0.99) (Figure 1) between the inhibition of iron absorption in humans and the amount of phytate. The iron absorption seems to increase at the point of intersection which corresponds to about 10 mg of inositol phosphate and this amount is approximately equivalent to one Fe atom per molecule of inositol hexaphosphate.

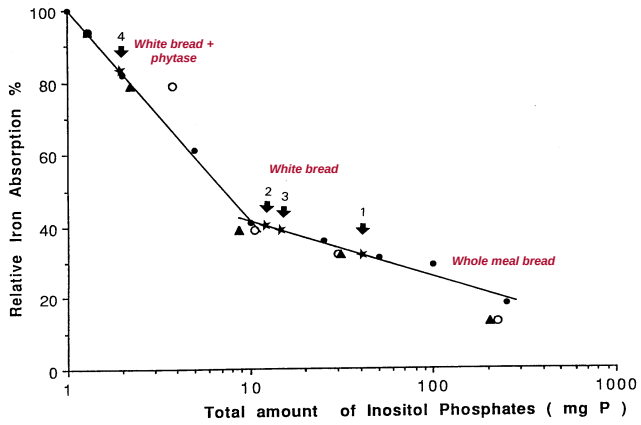
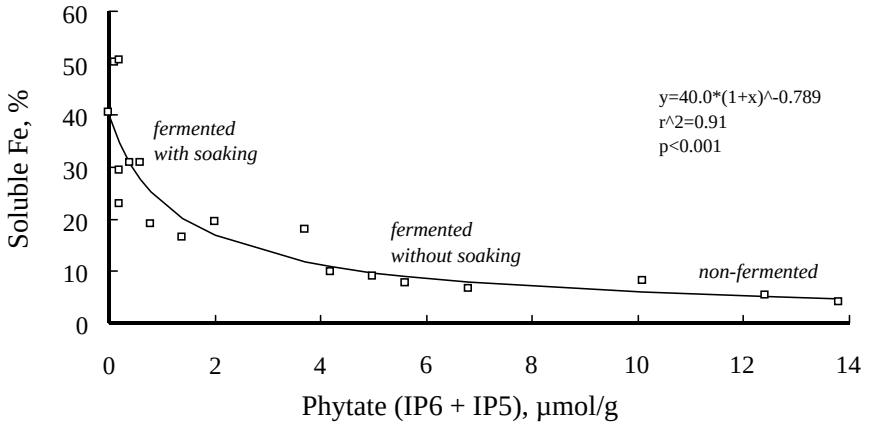


Figure 1. Iron absorption in relation to the content of inositol phosphate (IP_3 - IP_6) in different types of bread. (Brune et al. 1992).

Soaking and fermentation

Phytases, which hydrolyze phytate into lower inositol phosphates, are present in most cereals and are believed to be activated during the germination and fermentation processes. Phytate was shown to be completely hydrolyzed after fermentation of germinated white sorghum and, as a result, the amount of soluble iron was found to be strongly increased (Svanberg and Sandberg, 1988). Hydrolysis of phytate has also been reported in lactic acid-fermented maize (Lopez et al., 1983) pearl millet (Mahajan and Chauhan, 1987) and in germinated finger

millet (Udayasekhara Rao and Deosthale, 1988). Figure 2 shows the effect on the iron solubility (index of iron bioavailability), measured by an *in vitro* method, in different cereal gruels (non-tannin maize, sorghum and millet varieties). The gruels were prepared from flour that was either non-fermented or fermented with or without soaking prior to fermentation (Svanberg et al., 1993). A significant increase in iron solubility occurs when the phytate is degraded by more than 50%. The fermentation process can provide optimal pH conditions for degradation of phytate. The pH of the unfermented gruel is about 6.5 and reaches pH 3.6 once fermentation has been completed. The pH interval 5.0-4.5, believed to be optimal for cereal phytases, is thus achieved during the fermentation process.



Source: Svanberg *et al*. 1993

Figure 2. Relationship between soluble iron and amount of phytate (as inositol hexa- and pentaphosphate) in gruels of non-tannin cereals, either non-fermented or fermented with or without soaking in water prior to fermentation.

In order to completely degrade the phytate, sufficient time is needed within the optimum pH range, which is obtained by initial soaking of the flour in water for about 24 h. During soaking, the pH of the water/flour slurry slowly decreases to about 5.0 after which the fermentation can be initiated by adding a starter culture. This modification of the traditional method increases the amount of soluble iron up to ten-fold. Similar findings are reported in human studies (Hurrel et al., 2003). Iron absorption from wheat porridge increased 12-fold, and a 3–5-fold increase in absorption was observed on dephytinization (incubation with commercial phytase) of rice, wheat-soy, and maize porridges. However, phytate degradation of low-tannin sorghum porridges increased iron absorption only 2-fold, and no improvement in iron absorption was observed on dephytinization of high-tannin sorghum.

Phytase expressing yeasts

However, the phytase activity existing in cereals is being inactivated during cooking of the gruel that precedes the fermentation step. Therefore any phytate degradation needs to be mediated by phytase produced by the fermentation microflora. Phytate-degrading enzymes exist in yeasts, mycelia fungi and potentially in rare cases of lactic acid bacteria isolated from sourdoughs (Lopez et al., 2000; Sandberg and Andlid 2002; de Angelis et al., 2003).

In a model study (Hellström et al., 2007) of a lactic acid fermented maize porridge we tested whether a yeast with known high capacity to express phytases (BY80, a strain of *S. cerevisiae* genetically changed towards high constitutive phytase expression) (Veide and Andlid 2006), and an enzyme solution of commercially available *Aspergillus* phytase (no cells) would

degrade the phytate content. We also included a strain of *Lactobacillus pentosus*, with or without the high-phytase
yeast. In short, the data show that it is possible to partially degrade the phytate present in Togwa using the yeast BY80 (groups 2 and 3, Figure 3) and completely degrade the phytate with added pure *Aspergillus* phytase (fourth group of bars, Figure 3).

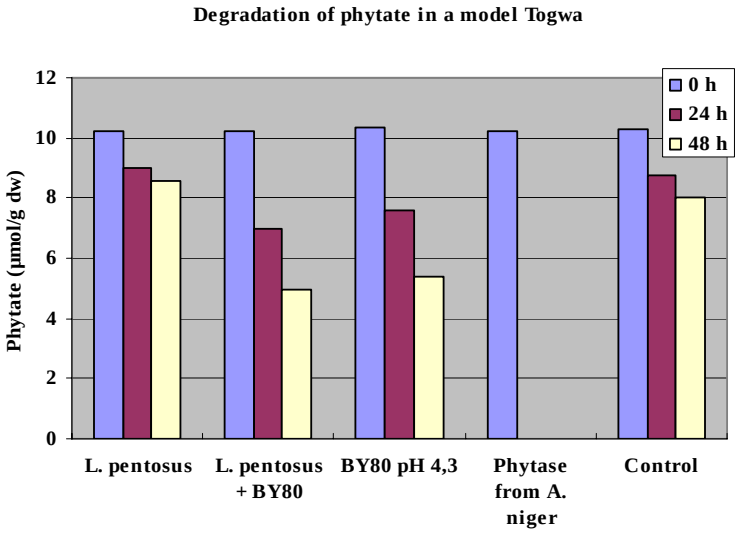


Figure 3. Phytate concentration in model Togwa at 0 (blue), 24 (red) and 48 (yellow) hours of fermentation, using different starter cultures as indicated.

The incomplete degradation of phytate indicates that the conditions were not optimal for the yeast used or that the yeast phytases were present inside the rigid and selective yeast cell wall which in many environments would be a hinder for interactions between substrate and enzyme. The results, however, suggest a high potential for improvements by developing yeasts originally isolated from and adapted to the Togwa ecosystem. Screening for such yeasts was done on Petri dishes with the use of BCIP, 5-Bromo-4-Chloro-3-Indolyl phosphate, to indicate phytase activity from a colony. Positive colonies stain blue and Figure 4 A & B shows a phytase producing yeast strain isolated from Togwa with extracellular activity.

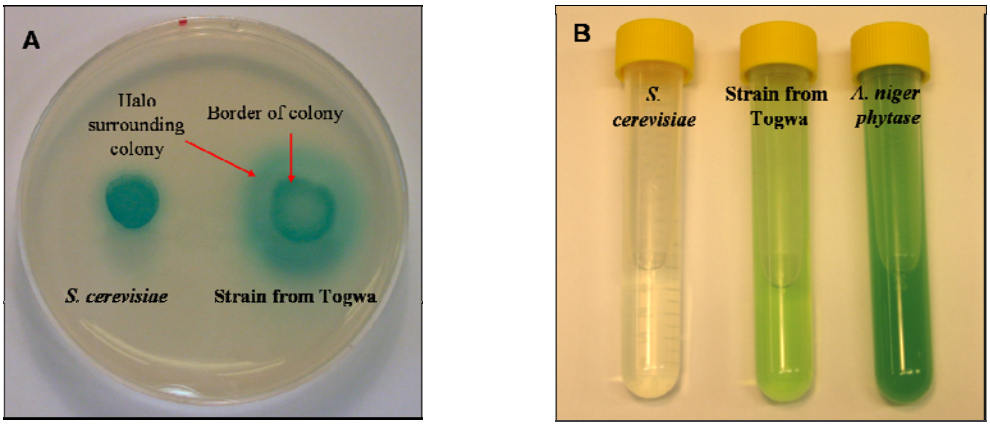


Figure 4. A. Rapid screening of phosphatase activity. Right colony shows extracellular activity. **B.** Activity detected in the cell-free supernatant of a strain isolated from Togwa.

Scalding and sour-dough fermentation

Scalding and sour-dough fermentation of bread are two traditional bread-baking techniques that are of great importance for phytate degradation. In these processes, indigenous phytases are activated. (Larsson and Sandberg, 1991). A phytate reduction of 96-97% occurred in bread made with 10% sour dough (pH 4.6) or in breads in which the pH had been adjusted by addition of scalded rye bran and lactic acid, resulting in a pH between 4.4 and 5.1 in both dough and bread.

Effect of polyphenol degradation

There seems to be no or little effect on the iron availability due to reduced levels of tannins as an effect of lactic fermentation of tannin-rich cereals. *In vitro* studies (Svanberg et al., 1993) showed that polyphenols in fermented gruels of high-tannin sorghum strongly inhibit iron solubility. The amount of soluble iron was about 13% after complete phytate degradation, while in non-tannin sorghum the amount of soluble iron was about 40% with the same treatment.

Oxidation of polyphenols with PPO in fruit extracts

In a recent study (Matuschek and Svanberg 2005), we incubated dephytinized high-tannin sorghum flour with crude water extracts from pear, banana or avocado all containing polyphenol oxidase (PPO) activity. The *in vitro* availability of iron increased significantly (Figure 5) from 50 to 170%. Furthermore, incubation with fruit extracts decreased the amount of phenolic compounds by 25, 43 and 50% for pear, banana and avocado extract, respectively. The available iron showed a high inverse relationship with the phenolic content (r = -0.956).

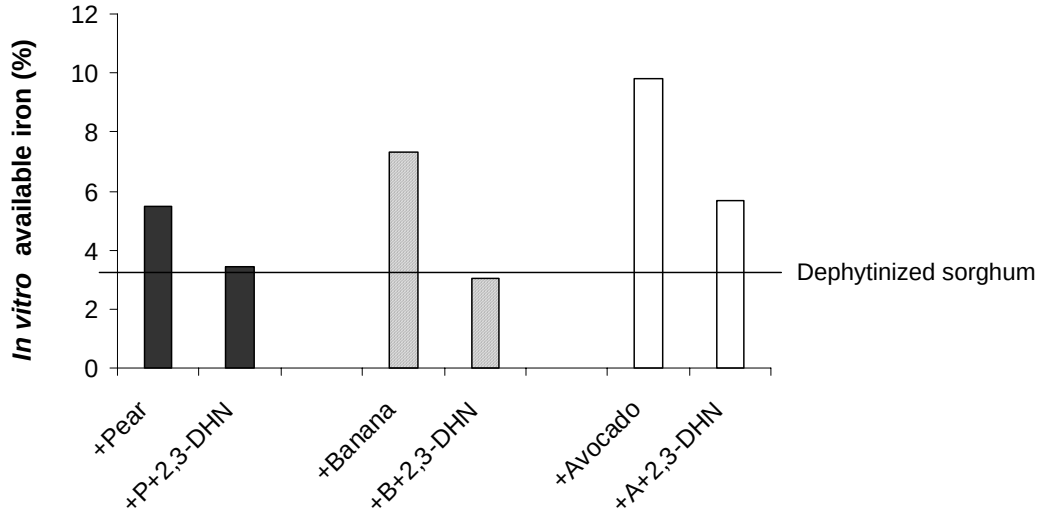


Figure 5. Effect of incubation with extracts from pear, banana or avocado, respectively, on *in vitro* accessible iron in dephytinized high-tannin sorghum. Fruit extracts containing 30 mM 2,3-dihydroxynaphthalene (2,3-DHN) were used as control samples. Mean values ±SD of triplicate samples are shown (from Matuschek and Svanberg 2005).

CONCLUSIONS

Traditional cereals are important sources of iron; however, the bioavailability of this iron is low due to presence of anti-nutritional factors such as phytate and phenolic compounds. Household-level food processing techniques can though be modified to substantially degrade such anti-nutritional components and thereby improve the iron bioavailability. In quantitative

terms, this means a diet of ‘low iron bioavailability’ can be changed into a diet of ‘intermediate to high iron bioavailability’, which otherwise only could be achieved by including generous quantities of iron absorption promoters, such as meat or foods containing large amounts of ascorbic acid.

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