Iron, Zinc and Phytic Acid in Rice from China:

Wet and Dry Processing towards Improved Mineral Bioavailability

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Contents

Abstract

Rice and rice products supply two thirds of Chinese people with their staple food. Mineral deficiencies, especially of iron and zinc, are prevalent in China, and are caused by insufficient intake and poor bioavailability. Rice and rice products contribute more than 50% of the antinutrient phytic acid consumed in the average diet, which has a significant negative impact on mineral bioavailability. This thesis reports studies of dry and wet rice processing methods on levels and in vitro solubility of minerals, and of phytic acid with the aim to contribute to the improvement of the bioavailability of minerals in rice-based diets.

Iron levels in 56 rice varieties from China ranged 9-45 mg kg^{-1}, zinc 13-39 mg kg^{-1}, and phytic acid ranged between 7.2-11.9 g kg^{-1}. Molar ratios of phytic acid to minerals indicated that the bioavailability of minerals in brown rice will be < 4%.

Dry processing by abrasive milling showed that short-, medium- and long-grain varieties suffer different losses of yield during abrasive removal of the bran. Phytic acid and iron are gradually removed at similar rates, while zinc is retained better. This results from the different spatial distribution: the highest concentration of iron and phytate was observed in the testa and the interface of embryo and perisperm, whereas zinc was concentrated in the endosperm and in the embryo. This offers limited opportunities for optimization of abrasive milling for maximum phytate removal, at minimum losses of yield and minerals.

Wet processing (soaking under various conditions, phytase application, fermentation, and germination) revealed that physical structure (intact or damaged kernels) affects migration of components into the water phase, and that phytase and acidic conditions favour the removal of phytic acid. Simultaneously, minerals may be lost by diffusion into the water phase. Although molar ratios of phytic acid to iron and zinc indicated higher in vitro solubilities, these improvements were hardly significant. An exception is the promising dephytinization of bran by phytase treatment while extracting significant levels of minerals. This treatment should receive further investigation because it potentially offers a way to valorize the by-product bran into a concentrate of soluble minerals.

Rice products available in supermarkets in China were investigated for their levels of phytic acid and minerals, and mineral solubility. Brown rice had much higher calcium, iron and phytic acid levels than white rice, but mineral solubility in both types of products was equally low. Rice noodles and rice crackers had low phytic acid and varying mineral contents and high solubility of especially calcium. In all product categories - primary and intensively processed - residual phytic acid levels were too high and mineral levels insufficient to be considered as good dietary sources of minerals. There is scope for better rice-derived noodles or crackers that are dephytinized, and preferably fortified with minerals.

The implications for the food chain are discussed. In the short term, dietary intervention by mineral supplementation and food dephytinization and fortification should be considered. On the longer term, selection and breeding of new rice varieties attuned to local agricultural practice, and nutrition education of the general public to stimulate a balanced diet and healthy lifestyle, will be sustainable solutions for the future.

Foreword

I started my march towards the PhD at Wageningen University, The Netherlands in October 2002. Study and research for the Doctor's Degree was an important experience for me in my past times. I'd like to express my appreciation to many persons since without their help, support and contribution it would have been impossible for me to finish this doctor's thesis.

During this relatively long period, I myself did not only obtain scientific knowledge, and research experience from my supervisors, but I also learned how to communicate well with others and how to deal with difficult situations. I would like to thank Professor Han Beizhong (I use the Chinese way for Chinese). It is Professor Han who recommended me as a candidate for Sandwich PhD to Rob Hamer (my promotor) and Rob Nout (my co-promotor), although it started just as a casual joke. Sometimes, I think I would have another kind of life if I had not made that joke with Professor Han and did not start my PhD study in 2002. I experienced a totally different life than in China during my first stay at Wageningen for three months in 2002, and this was the result of Han's recommendation and introduction. I tried my best to follow the recommendation of Han, which gave me a significant improvement of my spoken English, and more self-confidence in that short time.

I am deeply indebted to Professor Rob Hamer, and Dr. Rob Nout. They, together, provide me the PhD position and a strong supervision. I have learned a lot on methodology of research from my discussions with Professor Hamer. The skills of designing experiments, data analysis, procedure for writing papers, and experimental techniques will benefit me in future work. I am really lucky to have had the chance to be a student of Rob Hamer and obtain his supervision on the scientific aspect.

Rob Nout, my daily supervisor, really gave me a lot of daily supervision and support, not only in the scientific field, but also in daily life. I learned a lot from him. Besides lots of techniques concerning research design and experimental methods, I also obtained many others. "Can you follow me?" this simple sentence always reminds me to pay some more attention on my new students since new ones need more care. His attitude to students stimulated me to be closer to my students, not only about their study and research, but also in their daily life. I was also supported by him during my work in China, application of new software, and the improvement of my English. I enjoyed the talking about the differences between persons and culture of Dutch and Chinese with him. My special thanks go to Frances (Mrs. Nout). I enjoyed many times a meal at their house and walking or riding with the family. I still clearly remembered my first bicycle "tour" in Wageningen, which was organized by Rob Nout and Frances. Their elaborate explanation made my life in Wageningen simple, their kindness and encouragement alleviated my homesickness.

The INREF "From natural resources to healthy people: food-based interventions to alleviate nutrient deficiencies" committee, namely Dr. Maja Slingerland and Dr. Tjeerd-Jan Stomph always supported me during my study. And Dr. Ellis Hoffland encouraged me many times for my research. My thanks also extend to their families. Thank you for your pleasant dinners. Thanks to my colleagues in INREF project, Jiang Wen (plant modeling), Gao Xiaopeng (plant nutrition) and Qin Yu (human nutrition), my knowledge was extended beyond food sciences. I was always pleasant to talk with you and eat Chinese dishes together in Wageningen.

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Finally, my special thanks to my husband, Dong Zhongxin, and my son, Dong Hejia (Billy). During the past five years, I went abroad for ten times. Without their understanding and supporting, I could not have persisted to reach the goal. Thank you very much, my husband and my son!

梁建芬
Jianfen LIANG
Wageningen, October 2007

Chapter 1

General introduction

INTRODUCTION

Rice is the most important staple food crop in the world. More than half of the world population use this cereal as staple food, mainly in developing countries. In Asia, rice is the main dietary source for energy, protein, thiamine, riboflavin, niacin, iron and calcium (Juliano 1997). Because of the importance of rice, FAO named the year 2004 'International Year of Rice' and the theme of the year was 'rice is life'.

For consumption purposes, rice kernels are composed of two parts, the inedible hull and edible brown rice. Brown rice (dehulled rice) is composed of surface bran (6-7% by weight), endosperm (≈ 90%) and embryo (2-3%) (Chen et al. 1998). White rice, the main consumption product, is referred to as polished or whitened rice when 8-10% of mass (mainly bran) of the outer part has been removed from brown rice (Kennedy et al. 2002a).

RICE IN CHINA

In China, rice, wheat and maize are the most important three cereals produced. In contrast to maize, having the highest production among cereals, but going into animal feed or used as industrial raw material, 90% of rice is eaten directly as food. Figure 1 represents the production of rice, quantity of rice for food use and area used for rice and cereals cultivation in China during 1990-2004 (FAO 2006; NBSC 2007). It is shown that the quantity consumed in China and energy supplied by rice decreased gradually since 2000. This trend can be explained by the increase of animal-derived foods in the diet in the whole country (Wang 2005). Actually, rural regions are an exception: consumption of rice in rural regions in 2002 was at the same level as in 1992 (Wang 2005).

Rice is cultivated widely in China. According to the different natural conditions and

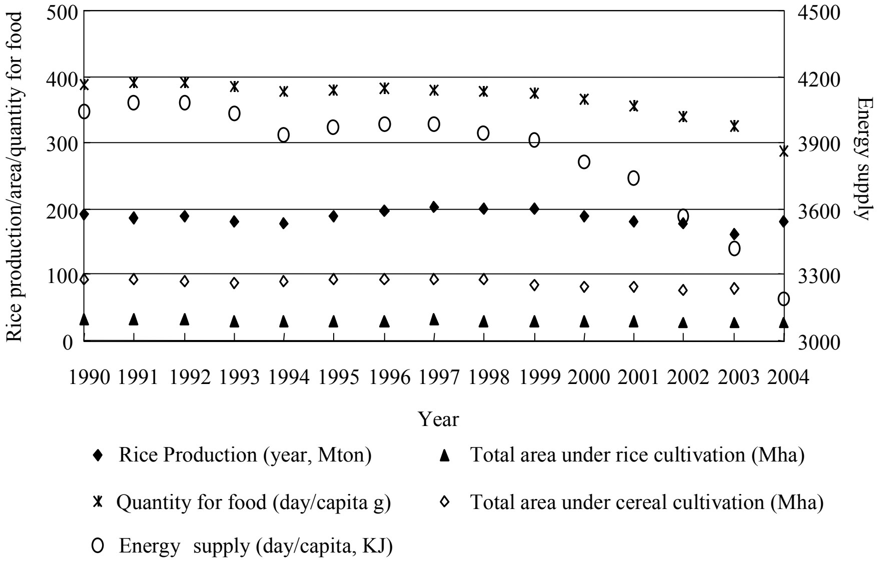
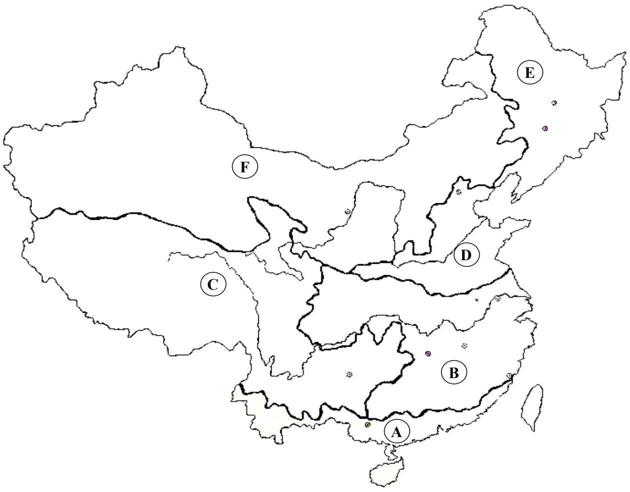


Figure 1: Rice production, growing area and food supply in China

agrotechnological infrastructure, six rice growing regions can be distinguished as shown in figure 2 (Zhou 1993).



A: Southern China; B: Mid China; C: South West Plateau; D: Northern China;
E: North East China; F: North West Dry Regions

Figure 2: Rice growing regions in China

In China, rice is the staple food for about two thirds of the population. During the period of 1997-2001, rice products supplied about 30-40% of the dietary energy (Kennedy et al. 2002b). In 2002 and 2003, it was reported that intake of rice and rice products was 238 and 215 g/capita/day, respectively, which represented 35% of the total energy intake (FAO 2006; Wang 2005). In the rural areas in 2002, the intake of rice and rice products was 246 g/capita/day, which was 3% higher than the average national level. Rice also supplies some important micronutrients, e.g. iron, zinc, calcium and some vitamins. Total iron supply was estimated at 22.4 mg/capita/day in 1992, and with changes of food consumption, intake of iron slightly increased to 23.1 mg/capita/day in 2002. However, iron deficiency related anaemia did not decrease with increase of iron intake, and the prevalence of iron deficiency anaemia in pregnant and lactating women was 30.4% in 2002 (Wang 2005). Although rice products are an important source of micro nutrients in China, data on bioavailability of minerals in these products are scarce. Especially for those who live in rural southern China, rice plays an even more important role in the diet. Because of their food habits and preferences, people in China seldom consume brown rice. Brown rice is a light brown coloured product obtained after removal of the husk of rice kernels using a hulling machine. Edibility of brown rice is considered poor because of its high fibre content and dark colour. Processing into white rice will lead to better acceptability, although at the same time, nutrients are also lost, especially protein, vitamin B and minerals (Kennedy et al. 2002b). As a rough estimate, about 10 million tons of rice bran are produced as a by-product of white rice processing in China (Gibson 2006). Long term consumption of white rice and restricted supply of animal food contribute to nutrient deficiencies, especially of minerals; this situation affects particularly the rural people.

The biggest nutritional shortfall of rice as a staple food is its low content of protein, iron, iodine and vitamin A. In the world, 3.5 billion people were reported iron deficient (Kennedy et al. 2002b). Iron deficiency anaemia is very serious in China. It was reported that about 20% of the Chinese have iron deficiency anaemia with 30% of very young (<2 years old) and old (>60 years old) people. The prevalence in rural areas is significantly higher than in cities. During the period 1992-2002, the dietary structure improved with the increase of consumption of animal-derived foods. However the incidence of iron deficiency anaemia slightly increased in the rural area (Wang 2005).

Table 1 illustrates the role of white and brown rice as dietary sources of minerals and vitamins (Kennedy et al. 2002a).

Table1: Amount and percent of RNI (Recommended Nutrients Intake per Day) of micronutrients from rice intake (300g Male /250g Female)

		Calcium	Iron	Thiamine	Riboflavin	Niacin	Zinc
Male							
White rice	mg	20.9	1.4	0.1	0.11	2.8	2.9
	% RNI	2	5	12	9	17	21
Brown rice	mg	78.9	4.2	0.8	0.09	10.5	4.9
	% RNI	8	15	67	7	66	35
Female							
White rice	mg	17.4	1.16	0.1	0.1	2.32	2.4
	% RNI	1.7	2	10.5	7	17	12
Brown rice	mg	65.9	3.49	0.67	0.1	8.75	4.1
	% RNI	7	6	61	8	63	20

This table shows that, based on literature data, white rice will supply 1.7-5% of calcium and iron, 9-17% of the vitamins and about 21% of the zinc RNI. Whereas these values are rather low, the present thesis (Chapter 6) will show that rice-based consumer products actually supply higher levels of minerals, corresponding to 38-39% of the RNI for iron and zinc. These percentages significantly increase if brown rice would be consumed instead of white rice. But then also more phytic acid will be consumed and this will negatively affect the bioavailability of some nutrients as will be discussed in the following part.

MINERALS AND PHYTIC ACID IN BROWN RICE

In general, deficiency of minerals in rice is due to their low concentration and the presence of inhibitors. Earlier studies showed that minerals in rice occur at low levels and that they are influenced by many factors. For example, iron levels in rice differ with growing regions. Whereas high iron levels (26 mg kg^{-1}) were found in Australian rice, Korean rice only had 7 mg kg^{-1}. In other countries, iron levels in brown rice were 22 mg·kg^{-1} for Indian rice and 12 mg kg^{-1} for rice from Vietnam and the Philippines. On the other hand, zinc shows relatively little variation around 20 mg kg^{-1}, reported from several countries (Doesthale et al. 1979; Kim et al. 2004; Lee et al. 1997; Phuong et al. 1999; Villareal et al. 1991). The knowledge about minerals in rice from China is still scarce.

The distribution of minerals in rice kernels is not uniform. About 50% of the mineral content is located in the bran layer and 10% in the embryo; both will be removed when producing white rice. White rice only contains 28% of the total ash of brown rice (Hunt et al. 2002), ash content being an index for mineral content.

Rice also contains phytic acid, a potential inhibitor of some important minerals such as iron and zinc, at average levels of about 120 mg 100g^{-1} in brown rice. Contents of phytic acid in milled white rice varied with a wide range from 55-183 mg 100g^{-1} depending on varieties and locations (Kennedy et al. 2002a; Ma 2007). The chemical description for phytic acid is myo-inositol 1,2,3,4,5,6 hexakis dihydrogen phosphate or myo-inositol hexakisphosphate (IP6). The structure of phytic acid and its chelator action are shown in figure 3.

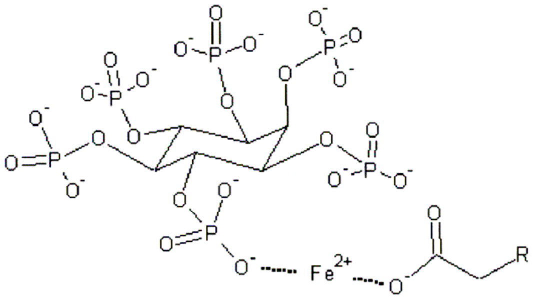


Figure 3: Phytic acid chelating with iron

Phytate occurs in cereals as a mixed mineral salt of phytic acid that has a function in chelating and storing phosphorus in the seed. It represents more than 80% of total phosphorus in cereals and legumes (Centeno and Viveros 2001; Oatway et al. 2001).

Phytic acid received attention as an anti-nutritional factor (ANF) in cereals and legumes. It can complex minerals and proteins, and influence their solubility and bioavailability. Intake of phytic acid might be one important factor causing deficiency of essential nutrients. For example, in some areas of China, iron deficiency anaemia is prevalent in spite of an intake of iron of 177% of the RNI published by WHO (World Health Organization) (Du et al. 2000). As iron intake is high, low bioavailability is considered a major factor for iron deficiency anaemia (Ma 2007). In China, foods of plant origin are the major sources of minerals, which provide most of dietary intakes of iron (76.9 vs 86.1%) and zinc (61.2 vs 76.9%) for urban and rural residents (Ma 2007). Although plant foods supply considerable amounts of minerals, intakes of phytic acid are also very high, 781 mg/day and 1433mg/day for urban and rural residents, respectively. This pattern may play an important role in minerals deficiency in China (Du et al. 2000; Ma 2007). Therefore, the effect of phytic acid in plants on the bioavailability of iron and other (micro) nutrients requires serious attention.

In recent years, also some beneficial health effects of phytic acid have been reported. Phytic acid is an effective antioxidant (Cornforth 2002). Phytic acid has even been proposed as a cure against colon cancer (Janab and Thompson 2002). It has also been reported that it can reduce the toxicity of some heavy metals due to its strong chelating capacity (Persson et al. 1998).

This thesis focuses on the negative effects of phytic acid, since deficiency of minerals is one of the biggest problems in China.

IMPROVING THE BIOAVAILABILITY OF MINERALS IN RICE

As mentioned, the bioavailability of minerals in rice is low. Three approaches, namely increasing the concentration of minerals, decreasing the concentration of mineral inhibitors, and increasing the levels of enhancers of mineral uptake, could improve the bioavailability of minerals.

Increasing the concentration of minerals in rice and rice products

Numerous research activities both in the fields of plant breeding and of food processing have been carried out, aimed at achieving relatively high concentrations of minerals in rice and rice products. Studies on the differences of iron/zinc and phytate phosphorus concentration in rice indicated that there are 4-fold differences in mineral levels among rice genotypes, which suggests that there is a potential to increase the concentration of these micronutrients in rice grain with genetic technology (Raboy and Bouis 2002; Welch and Graham 2004). Modern biotechnology could improve mineral levels in rice (Glahn et al. 2002; Graham et al. 1999; Lucca et al. 2001). Most of this research is still in an experimental phase. At present, optimization of rice processing could be an effective and practical way to increase the levels of minerals in rice products. Optimization of milling and polishing procedures could help reduce the inevitable loss of minerals, while parboiling and fortification could help increase mineral contents in final rice products (Mannar and Gallego 2002; Mohapatra and Bal 2004; Singh Gujral et al. 2002).

Decreasing the levels of mineral inhibitors in rice

Phytic acid and fiber are major mineral inhibitors in rice. Phytic acid is an anion at physiological pH (7.35-7.45), and forms insoluble complexes by chelating minerals and proteins. As a result, the bioavailability of these (micro)nutrients will be decreased (Sandberg et al. 1999). Mechanical processing, such as milling and polishing and enzymatic degradation of inhibitors could help remove phytic acid and fiber (Gibson et al. 2000; Resurreccion et al. 1979).

Increasing the levels of mineral enhancers

Increasing the levels of enhancers of minerals could also improve their bioavailability. Individual minerals may be enhanced by different compounds (Gibson et al. 2000). For example, ascorbic acid is one of the enhancers of iron uptake. The bioavailability of non-heme iron improves when molar ratios of ascorbic acid to iron are 0.8:1 and maximum at 1.6:1 (Glahn et al. 1999). However, ascorbic acid has not been shown to influence the bioavailability of zinc (Gibson et al. 2000).

STRATEGIES TO IMPROVE THE BIOAVAILABILITY OF MINERALS BY DECREASING THE LEVELS OF PHYTIC ACID

As indicated above, decreasing the levels of phytic acid may be expected to improve the mineral bioavailability in rice. Dry and wet processing, such as milling, soaking, germination and fermentation have been advocated for the reduction of phytic acid. Below, a few examples are mentioned of data obtained previously by other investigators.

Milling: By abrasive removal of 5% of the total grain weight, total ash and phosphorus were lost to the extent of 40%, calcium 36%, iron 54%, and zinc by 17% (Doesthale et al. 1979). Loss of phytic acid during milling was about 70% (Tabekhia and Luh 1979).

Soaking: The content of IP6+IP5 (one phosphorus in IP6 was removed by hydrolysis) in rice flour was reduced by 60% and 65% after 1 and 6 h soaking in water, and almost completely after 12 h soaking, but this treatment only had little effect on iron, zinc and calcium levels (Perlas and Gibson 2002).

Cooking: The phytic acid concentration of brown rice and white rice was decreased by 24% and 65% respectively after cooking (Franz et al. 1980).

Fermentation: Natural fermentation can achieve a large reduction in phytic acid in rice flour by the action of bacterial as well as grain phytases. These reduce the hexa form of phytic acid into lower forms, which have a lower binding capacity for metals like iron and zinc (Agte et al. 1999). Results of fermentation on wheat products (bread) showed that it can improve in vivo bioavailability of minerals significantly (Turk et al. 2000).

Germination: Studies on legumes, millets, oat, and barley indicated that germination could decrease phytic acid with 30 to 90%, depending on the type of cereal (Agte and Joshi 1998; Archana and Kawatra 1998; Centeno and Viveros 2001). Decreased phytic acid levels resulted in a significantly improved bioavailability of micronutrients (Mamiro et al. 2001).

Phytase enzymes: Commercial phytase prepared from *Aspergillus oryzae* or *A. niger* may be used. They can facilitate phytate hydrolysis (Gibson et al. 2000).

In summary, among the approaches mentioned above, there are three different mechanisms of reduction of phytic acid.

1) Reduction by removal: milling belongs to this group. Reduction of phytic acid in this group depends on the distribution of phytic acid in rice kernels. Minerals will also be lost with decrease of phytic acid.
2) Reduction by diffusion: soaking and cooking belong to this group. Only diffusion of phytic acid into medium could explain the reduction of phytic acid since phytic acid has been shown to be heat stable (Henderson and Ankrah 1985; Mahgoub and Elhag 1998; Oatway et al. 2001). With diffusion of phytic acid, minerals will also be lost.
3) Reduction by degradation of phytic acid: fermentation, germination and application of phytase belong to this group. By the activity of endogenous or exogenous phytases, phytic acid is hydrolysed and degraded to lower inositol phosphates. These treatments could only affect contents of IP6, while having little effect on mineral levels.

In our study, these three approaches will be assessed to improve the availability of minerals in rice and rice products.

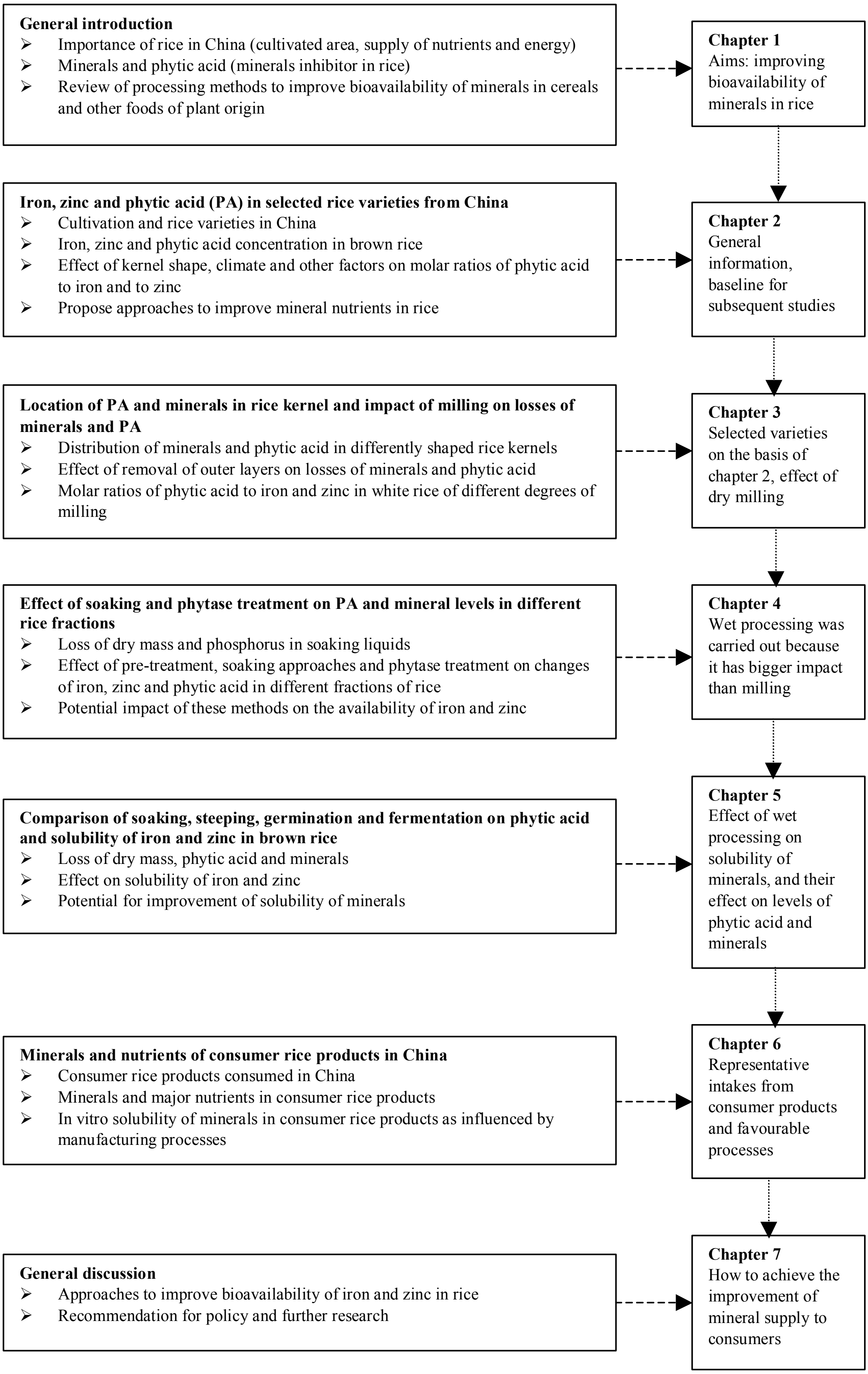
OBJECTIVES AND SCHEME OF THE THESIS

The objectives of this thesis are:

1) Investigate contents of minerals and phytic acid in rice from producing regions in China to obtain information about levels and ranges of minerals and phytic acid;
2) On the basis of this information, to investigate the distribution of minerals and phytic acid in rice kernels to support optimization of dry processing (milling);
3) Study the effect of wet processing, including soaking, germination, fermentation and phytase application on contents of minerals and *in vitro* solubility of iron and zinc.

OUTLINE OF THE THESIS

In order to achieve the objectives of the thesis, several studies were carried out. In total, 7 chapters in this thesis describe the results obtained. These chapters and their interaction are schematically depicted as follows.



General introduction
- Importance of rice in China (cultivated area, supply of nutrients and energy)
- Minerals and phytic acid (minerals inhibitor in rice)
- Review of processing methods to improve bioavailability of minerals in cereals and other foods of plant origin

Chapter 1
Aims: improving bioavailability of minerals in rice

Iron, zinc and phytic acid (PA) in selected rice varieties from China
- Cultivation and rice varieties in China
- Iron, zinc and phytic acid concentration in brown rice
- Effect of kernel shape, climate and other factors on molar ratios of phytic acid to iron and to zinc
- Propose approaches to improve mineral nutrients in rice

Chapter 2
General information, baseline for subsequent studies

Location of PA and minerals in rice kernel and impact of milling on losses of minerals and PA
- Distribution of minerals and phytic acid in differently shaped rice kernels
- Effect of removal of outer layers on losses of minerals and phytic acid
- Molar ratios of phytic acid to iron and zinc in white rice of different degrees of milling

Chapter 3
Selected varieties on the basis of chapter 2, effect of dry milling

Effect of soaking and phytase treatment on PA and mineral levels in different rice fractions
- Loss of dry mass and phosphorus in soaking liquids
- Effect of pre-treatment, soaking approaches and phytase treatment on changes of iron, zinc and phytic acid in different fractions of rice
- Potential impact of these methods on the availability of iron and zinc

Chapter 4
Wet processing was carried out because it has bigger impact than milling

Comparison of soaking, steeping, germination and fermentation on phytic acid and solubility of iron and zinc in brown rice
- Loss of dry mass, phytic acid and minerals
- Effect on solubility of iron and zinc
- Potential for improvement of solubility of minerals

Chapter 5
Effect of wet processing on solubility of minerals, and their effect on levels of phytic acid and minerals

Minerals and nutrients of consumer rice products in China
- Consumer rice products consumed in China
- Minerals and major nutrients in consumer rice products
- In vitro solubility of minerals in consumer rice products as influenced by manufacturing processes

Chapter 6
Representative intakes from consumer products and favourable processes

General discussion
- Approaches to improve bioavailability of iron and zinc in rice
- Recommendation for policy and further research

Chapter 7
How to achieve the improvement of mineral supply to consumers

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Chapter 2

Iron, zinc, and phytic acid content of selected rice varieties from China

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Abstract

Rice is the major Chinese staple food (per capita approx 250 g day^{-1}) and, as such, an important source of essential minerals. However, due to a number of factors the bioavailability of these minerals is limited. In this study, the variation of phytic acid, iron and zinc levels in 56 varieties of Chinese rice was investigated. The samples included in this study were collected in proportion to the importance of the rice growing regions in China.

Iron levels showed the biggest variation (9-45 mg kg^{-1}) and were not related with phytic acid content, or grain shape although growing locations were identified yielding higher (25.2 mg kg^{-1}) and lower (14.2 mg kg^{-1}) iron levels. Zinc showed a moderate variability (13-39 mg kg^{-1}), which was narrower than for iron, while broader than for phytic acid (7.2-11.9 g kg^{-1}). Zinc content is correlated (R^2 = 0.5; $p<0.01$) with phytic acid content, and shows a relation with growing region and kernel shape. Variation of phytic acid content is the least among the three components. Molar ratios of phytic acid to iron and zinc ranged from 15-105 and 27-67, respectively.

The results of the mineral contents and phytic acid content can be interpreted in terms of expected bioavailability. This study shows that the mineral bioavailability of Chinese rice varieties will be <4%. Despite the variation in mineral contents, in all cases the phytic acid present is expected to render most mineral present unavailable. We conclude that there is scope for optimization of mineral contents of rice by matching suitable varieties and growing regions, and that rice products require processing that retains minerals but results in thorough dephytinization.

INTRODUCTION

Rice is the primary food source for more than half of the world population. In Asia, it serves as the major source of energy, protein, thiamine, riboflavin, niacin, iron and calcium in the diet (Juliano 1997).

Rice is also the most important staple food in China. In 2004, the national production was estimated at 18.7 million tons (FAO 2006), with a per capita consumption of 251 g day^{-1}. This accounts for 30.4% of the supply of dietary energy, 19.5% of dietary protein, and 2.5% of dietary fat (Kennedy et al. 2002). Rice is also the major source of intake of micronutrients such as zinc, iron, and vitamins for the Chinese, especially those who live in the southern countryside.

Unfortunately, rice does not supply minerals adequately. It only has limited contents of iron and zinc, and moreover the loss of minerals, particularly of iron, during rice milling is high (Doesthale et al. 1979). In addition, rice contains phytic acid, which is the most important anti-nutritional factor impeding availability of divalent minerals. It forms complexes with mineral ions, such as Fe^{2+}, Zn^{2+} and Ca^{2+}, and ultimately affects their bioavailability (Gibson et al. 2000; Lucca et al. 2001; Mendoza 2002).

The bioavailability of minerals in rice could be improved by the selection of varieties with a high mineral content suitable for certain growing regions, by using plant breeding for high mineral content or low phytic acid content, or by processing methods that either improve the mineral content and/or its bioavailability (Gibson et al. 2000; Lonnerdal 2002; Welch and Graham 2004).

Although rice is one of the most important cultivated cereals and has a significant effect on the nutritional status of the Chinese people, research on rice in China has until now been focussed mainly on yield, macronutrients such as protein and starch, and sensory quality.

Brown rice is the raw material for several rice-derived products like white rice, germinated rice, and rice noodles. Therefore, in this study we concentrated on brown rice and its potential to serve as a raw material for further processing, as a first requirement to improve mineral availability.

The objectives of this paper are: 1) to investigate the variation of content of phytic acid, iron and zinc in selected Chinese rice varieties and 2) to analyse the effect of variety and rice growing region on these parameters.

MATERIALS AND METHODS

Collection of rice samples

Samples of 56 rice varieties (1 kg per variety) were obtained from different growing regions in China. These regions and their classification are based on climate, soil quality and agro technological infrastructure. Locations and numbers of varieties collected are shown in figure 1.

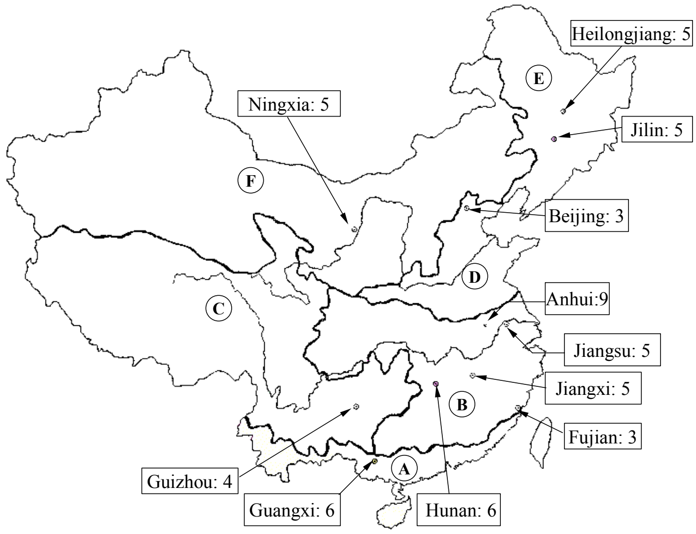

Figure1: Rice growing regions and locations where varieties were collected; with numbers of varieties collected

Currently, it is held that more than fifty thousand varieties of rice exist in China, of which about two hundred thirty are grown at a commercial scale. For this study, we collected fifty-six varieties of commercial relevance harvested in 2003 with the assistance of the Institute of Crop Germplasm Resources of the Chinese Academy of Agricultural Sciences, and the College of Agronomy and Biotechnology of the China Agricultural University.

The importance of the growing regions was a major criterion for sample collection. For example, 61.8% of the sampled varieties originate from regions A and B, since these two regions occupy 78% of total planting area of the whole of China (IRRI 2004). Five varieties of aerobic rice (origin Anhui) were included, as well as three "special" varieties, which are used for non-staple food preparations such as porridges.

Pre-treatment and processing of rice samples

Husks were removed with a laboratory-scale dehulling machine (JLGJ45, Zhejiang, China). The resulting brown rice was ground using two different protocols, depending on the purpose. For mineral analysis, the rice was ground using a Fritsch mill (Marius Instrumenten, the Netherlands; with a 0.5 mm sieve). For phytic acid analysis, samples were first dried at 70 ºC until constant weight, and were ground using a home blender (SG-280A, Shunde, China) to pass a 1 mm sieve. Ground samples were sealed and stored in closed containers at 4 ºC prior to analysis.

Measurement of kernel dimensions

The length and width of rice kernels were measured with a microruler (accuracy: 0.01mm). Of every grain, the maximum length and width were measured. Of each variety, 20 grains were measured, and the average was reported.

Iron and zinc contents

Iron and zinc contents were analysed with ICP-AAS (induced coupled plasma – atomic absorption spectrometry) (Marr et al. 1995). Samples were digested with HNO_3 (nitric acid)-H_2O_2 (hydrogen peroxide)-HF (hydrofluoric acid). Aliquots of 5.0 mL hydrofluoric acid and 5.0 mL nitric acid (65% w/w) were added to 0.3-0.4 g (±0.001g) of sample and were allowed to react overnight at room temperature (21ºC). Then, samples were evaporated until almost dry at 120ºC. Another 5.0 mL nitric acid (65% w/w) and three times 1.0 mL hydrogen peroxide (H_2O_2) were added and samples were digested in a microwave oven using a heating programme of subsequently 1 min at 100ºC, 5 min at 125ºC, 5 min at 150ºC and 10 min at 175ºC. Iron and zinc in filtered digested solutions were measured with ICP-AAS. All varieties were analysed in triplicates.

Phytic acid content

The China National Standard Analysis Method (GB/T 17406-1998) (Ma et al. 2005; Ministry of Health 1998) was used, based on extraction, separation on anion exchange resin, and spectrophotometric detection of the reaction product with ferric chloride ($FeCl_3$) and sulfosalicylic acid. All samples were analysed in triplicates.

Data analysis

Data were analysed by ANOVA and *t*-tests using the SPSS package (Sony DADC, version 12.0.1).

RESULTS AND DISCUSSION

Phytic acid, iron and zinc content in rice from China

Table 1 summarizes phytic acid, iron and zinc values determined in the Chinese varieties in comparison with data obtained in other regions.

In the Chinese set, the mean iron content is 18 mg kg^{-1}, which is lower than that found in Indian and Australian group B, but higher than Vietnamese and Korean varieties. The iron content in Chinese varieties ranged from 9 to 45 mg kg^{-1}. This variation is larger than that observed in the Vietnamese and Korean sets of rice samples. On the other hand, the set of Australian samples showed an even larger variation (5-67 mg kg^{-1}). As for zinc content, values obtained with Chinese rice were within the range of the other reported data. In our varieties, the variation of zinc content is smaller than that of iron content.

Table 1: Comparison of iron (Fe), zinc (Zn) and phytic acid (PA) content of Chinese brown rice with international literature data

	China		Vietnam		Australia[a]				Korea		India		IRRI[b]	
	(n = 56)		(n = 25)		A: (n = 90)		B: (n = 22)		(n = 68)		(n = 16)		(n =7)	
	(Dry Mass)		(Phuong et al. 1999)		(Marr et al. 1995)		(Kim et al. 2004)		(Lee et al. 1997)		(Doesthale et al. 1979)		(Villareal et al. 1991)	
			(Fresh weight basis)		(At 14% moisture)		(Not mentioned)		(Not mentioned)		(Not mentioned)		(Fresh weight basis)	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Fe (mg kg^{-1})	18.2	9.4 - 44.6	12.0	8.7 - 25.8	13	5 - 67	26	17 - 34	7.4	1.6 - 14	21.6	-	12.4	-
Zn (mg kg^{-1})	22.8	12.9 - 38.7	26.5	22.5 - 32.5	16	13 - 21	32	22 - 72	19.1	12.7 - 37.5	14.3	-	22.4	-
PA (g kg^{-1})	9.6	7.2 - 11.9	-[c]	-	-	-	-	-	12.6	8.6 - 17.6	-	-	-	-

[a]: two different literature data sets, labelled as A and B; [b]: International Rice Research Institute, Philippines; [c] data not published.

Phytic acid contents in Chinese rice also show some variation (7.2-11.9 g kg^{-1}). This variation is smaller than reported in Korean rice (8.6-17.6 g kg^{-1}). Phytic acid contents in rice from other countries were not available from published literature.

The differences between our samples and those from other studies could stem from both the type of samples included in these studies and the methods of analysis used. The latter differed among the reported studies. AAS (atomic absorbance spectrophotometry) was used for iron and zinc analysis in the Indian varieties, while energy-dispersed X-ray was used in the IRRI study (Villareal et al. 1991). Other studies (including this study) used the ICP-AAS method for mineral analysis. Procedures of pre-treatment of samples, their digestion and sensitivity limits of equipment might also affect the absolute levels of minerals reported in rice.

Molar ratios of phytic acid, iron, and zinc in brown rice varieties from China

The molar ratios of phytic acid to iron ([PA]/[Fe]) and zinc ([PA]/[Zn]), and iron to zinc ([Fe]/[Zn]) are presented in table 2.

Table 2: Molar ratio of phytic acid, iron and zinc in Chinese brown rice (n=56 varieties)

	[PA]/[Fe]	[PA]/[Zn]	[Fe]/[Zn]
Average	49.5 ± 16.0	42.9 ± 7.5	0.953 ± 0.354
Range	15 -104.8	27.0 -66.6	0.48-2.26
Correlation between the two components (R^2)	0.12	0.50*	0.24

* significant ($p < 0.01$).

In Chinese varieties, the mean value of [PA]/[Fe] in brown rice is 50. For comparison, this value is close to the ratio in brown rice reported previously (Wolters 1992). This ratio is much higher than that of wheat measured in India, which was only 5.45 (Grewal et al. 1999). The values of [PA]/[Fe] ranged from 15 to 105, indicating a big diversity. These ratios, even the lowest, are much higher than required to chelate more than 90% of iron (Subba Rao and Narasinga Rao 1983). According the model of Wolters (Wolters 1992), the availability of iron in Chinese brown rice would be 4.0-6.5% if we only take into account the effect of phytic acid. Since brown rice also contains other complex forming substances, such as dietary fibre, and does not contain enhancers of iron availability, the bioavailability of iron in brown rice in China will probably be even lower.

The mean value of [PA]/[Zn] in the Chinese varieties is 42.9, which is higher than that measured in brown rice in India (Subba Rao and Narasinga Rao 1983). There is slight correlation ($R^2 = 0.50$, $p < 0.01$) between the levels of phytic acid and zinc in brown rice. Values of [PA]/[Zn] in brown rice are also different from other cereals (Gruner et al. 1996).

Davies and Olpin mentioned that regardless of the absolute levels of zinc and phytic acid, the values of [PA]/[Zn] would be the major determinant of zinc availability. Marginal zinc deficiency in rats appeared at a value of [PA]/[Zn] of 10-15 (Davies and Olpin 1979). Similarly as calculated for iron, we can predict that the availability of zinc in Chinese rice is 2.4-3.9%, if phytic acid is the only factor taken into account. These data suggest that [PA]/[Zn] in Chinese brown rice must be reduced significantly to increase bioavailability of zinc.

The mean value of [Fe]/[Zn] in Chinese rice is 0.95, which is similar to other cereals (Gruner et al. 1996), although a higher ratio of 3.85 was reported elsewhere (Grewal et al. 1999). The mean value of [Fe]/[Zn] in our study is close to Doesthale's (0.80), who also reported that the level of iron in brown rice depends significantly on the level of zinc ($R^2 = 0.92$, $p<0.01$) (Doesthale et al. 1979). The latter correlation was lower in our study.

Effect of the morphology of rice kernels on the content of phytic acid, iron and zinc

The distribution of minerals and phytic acid in rice kernels is not homogenous. In general, the bran contains higher levels of zinc, iron, as well as phytic acid (Doesthale et al. 1979). Since the kernel size and shape could also relate to the ratio of volume to surface area, we analysed the possible effect of the morphology of rice kernel on the content of minerals and phytic acid.

The morphology of a rice kernel can be described by several parameters, such as length, width, and thousand-kernel weight (TKW). The properties of the collected samples are presented in table 3.

Table 3: Morphology and other properties of rice varieties from China

Variety	PL[a]	GR[b]	Length (mm)	L/W[c]	TKW[d] (g)	YBR[e] (%)	YWR[f] (%)	MC[g] (%)
II You 838	Anhui	B	6.4	2.5	19.13	80.2	70.3	8.1
Tesanai 2	Anhui	B	5.5	1.9	20.38	81.2	72.1	9.7
Xieyou 9019	Anhui	B	7.0	3.0	20.21	81.4	70.0	10.4
Zhongxian 898	Anhui	B	7.1	3.1	21.48	78.9	69.0	9.4
Han 65 (aerobic rice)	Anhui	B	5.2	1.8	16.25	76.8	58.1	8.4
Han 277 (aerobic rice)	Anhui	B	5.0	1.7	17.33	68.2	61.3	8.7
Han 297 (aerobic rice)	Anhui	B	5.5	2.0	19.96	77.0	69.9	6.6
Han 502 (aerobic rice)	Anhui	B	6.0	2.0	21.86	73.7	66.6	7.6
Han No.9 (aerobic rice)	Anhui	B	5.0	1.8	17.35	76.1	68.4	7.8
Jiamu Zaozhan	Fujian	B	7.4	3.5	20.13	79.7	68.2	9.8
Jinshanyou No. 1	Fujian	B	6.8	3.0	20.95	79.2	69.0	8.9
Weiyou 77	Fujian	B	6.7	2.6	22.19	80.8	66.5	8.7
Boyou 253	Guangxi	A	6.0	2.5	17.58	74.1	68.4	7.5
Qiuyou 1025	Guangxi	A	6.3	3.0	14.54	74.4	69.2	7
Qiuyougui 99	Guangxi	A	6.6	3.0	15.93	73.6	68.6	9.8
Teyou 63	Guangxi	A	6.3	2.4	22.41	70.9	63.4	7.5
Teyou 706	Guangxi	A	6.4	2.5	22.35	77.8	71.5	7

Variety	PL[a]	GR[b]	Length (mm)	L/W[c]	TKW[d] (g)	YBR[e] (%)	YWR[f] (%)	MC[g] (%)
Zhongyou 838	Guangxi	A	7.1	3.0	22.34	74.6	67.1	8
Bijing 37	Guizhou	C	5.1	1.8	18.24	75.2	68.9	7
Jinyou 431	Guizhou	C	6.3	2.4	19.21	66.3	59.5	7
Jinyou 527	Guizhou	C	7.4	3.0	23.59	70.3	62.2	7.5
Liangyou 363	Guizhou	C	7	3.0	20.95	71.8	65.5	7
Fushiguang	Heilongjiang	E	5.4	1.8	21.76	82.2	72.6	8.5
Hejiang 195	Heilongjiang	E	5.8	2.1	20.23	80.3	70.9	8.9
Xixuan No.1	Heilongjiang	E	5.7	2.0	19.70	81.1	72.6	10.4
Wuyoudao C	Heilongjiang	E	5.6	2.0	21.55	81.0	71.9	9.7
Wuyoudao No.1	Heilongjiang	E	5.7	2.0	18.95	79.2	70.2	9.7
Jinyou 207	Hunan	B	6.9	3.0	20.05	78.2	69.2	7.8
Jinyou 402	Hunan	B	7.4	3.1	22.15	81.5	67.3	8.6
R 981	Hunan	B	6.2	2.5	18.55	83.6	75.7	10.7
V 46	Hunan	B	6.2	2.2	23.39	79.3	70.6	7.8
Xiangwanxian No.11	Hunan	B	6.8	3.1	19.87	79.1	68.8	8.8
XiangzaoxXian No.31	Hunan	B	6.4	2.8	17.11	81.0	70.3	9.9
Nanjing 40	Jiangsu	B	5.2	1.7	21.15	84.7	76.2	7.2
Nanjing No.16	Jiangsu	B	7.3	3.2	21.18	74.6	68.8	7.4
Wuyujing No.3	Jiangsu	B	4.8	1.6	20.67	83.3	75.5	7.4
Wuyujing No.7	Jiangsu	B	5.0	1.6	20.99	83.5	76.4	6.6
Zhenjing 866	Jiangsu	B	6.7	2.9	20.30	75.2	68.9	6.2
Ganwanxian 30	Jiangxi	B	7.5	3.4	19.00	72.0	64.2	6.5
Ganzaoxian 49	Jiangxi	B	7.1	3.3	18.16	72.0	64.2	10.4
Jinyou 71	Jiangxi	B	6.7	3.1	17.16	70.0	61.7	7.6
Jinyou 402	Jiangxi	B	6.7	3.1	17.38	72.7	62.5	7.3
Zhongyou 752	Jiangxi	B	6.5	3.4	15.22	70.2	62.5	7.5
Changbai No.9	Jilin	E	5.2	1.7	21.89	84.8	76.2	8.9
Fengyou 307	Jilin	E	5.0	1.8	18.49	82.6	74.9	9.6
Qiuguang	Jilin	E	5.2	1.8	19.26	83.5	76.1	8.8
Tong 35	Jilin	E	5.2	1.8	20.76	83.1	74.7	9.5
Tong 95-74	Jilin	E	4.9	1.7	19.17	83.2	75.0	8.8
Ningjing 22	Ningxia	F	5.0	1.7	19.22	83.3	74.2	10.6
Ningjing 23	Ningxia	F	5.3	1.7	20.60	82.8	74.4	8.1
Ningjing 24	Ningxia	F	5.8	2.0	21.28	81.6	73.1	8.7
Ningjing 27	Ningxia	F	5.2	2.1	15.94	80.4	73.1	7.9
Ningjing No.5	Ningxia	F	4.9	1.7	19.21	81.4	72.1	9.6
Heinuomi 1568[h]	[i]	D	5.2	2.1	14.88	66.1	[j]	7.5
Jupei Xiangnuo 1574[h]	[i]	D	4.8	1.7	15.81	74.1	64.8	5.5
Tianhongmi 1571[h]	[i]	D	4.6	1.9	8.13	67.5	[j]	7.5

[a] planting location; [b] growing region as shown in Figure 1; [c] ratio of length to width; [d] thousand kernel weight (brown rice); [e] yield of brown rice from paddy; [f] yield of white rice from paddy; [g] moisture content of brown rice; [h] special varieties; [i] collected from the Chinese Academy of Agricultural Sciences; [j] impossible to obtain white rice from brown rice due to too small kernel size.

According to the Industrial Standard of the Ministry of China Agriculture (CHISA 2002), rice kernel length (L) can be distinguished into three groups. These are long (>6.5

mm), medium (5.5-6.5 mm) and short grains (<5.5 mm). *Indica* rice (in China referred to as *Xian* or *Hsien*, long grains) is dominant in the south, while *japonica* rice (*Jing* or *Keng*, short grains) is widely planted in the north. In addition, aromatic, glutinous and other special types of rice are appreciated (IRRI 2004); these are not used for milling and noodle making but for special dishes such as porridges. The rice varieties collected in this study represented the three groups (long, medium and short grains) by 33%, 29% and 38% respectively. The longest kernel was 7.5 mm, and the shortest was 4.6 mm. The shape of the kernel can also be characterized by the ratio of length to width. These ratios varied from 1.7 to 3.5. The TKW of brown rice ranged from 15.2 to 23.6 g, except for the special varieties. The yield of brown rice from paddy ranges at 66.1-84.8%, and the yield of white rice from brown rice at 58.1-76.2% with the exclusion of the special varieties. The moisture content of the seeds was lower than 11%. This indicated that the Chinese rice varieties differ greatly in morphology and processing properties.

Phytic acid, iron and zinc levels in brown rice did not show significant relation with TKW. On the other hand, phytic acid, iron and zinc levels had a significant relation with the ratio TKW/L at the 0.05 level. This ratio is a shape factor, instead of TKW alone. So, these results further confirmed that the shape of the kernels has a significant effect on the levels of phytic acid, iron and zinc in brown rice. We also observed a slight correlation between length of brown rice kernels (x [mm]) and yield factors (Y [%]) as follows:

$$Y \text{ (yield of brown rice)} = -2.5225x + 93.143 \ (R^2 = 0.202)$$
$$Y \text{ (yield of white rice)} = -2.8043x+86.25 \ (R^2 = 0.2644)$$

The relation of phytic acid, iron and zinc levels with the kernel length is presented in table 4 and shows that the mean value of phytic acid content tends to increase with kernel length, although not statistically significant. Notwithstanding, phytic acid contents related significantly ($p<0.05$) with the ratios of kernel length/width (L/W) which were shown in table 3. At L/W<2, mean value of phytic acid was 9.1, whereas L/W>2 had phytic acid mean value of 9.9.

Table 4: Phytic acid (PA), iron (Fe) and zinc (Zn) in short-, medium-, and long-grain rice

Kernel shape	Length (mm)	Range (mm)	PA (g kg^{-1})	Range of PA (g kg^{-1})	Fe (mg kg^{-1})	Range of Fe (mg kg^{-1})	Zn (mg kg^{-1})	Range of Zn (mg kg^{-1})
Short-grain	5.1±0.2	4.9-5.4	9.2±1.2 a	7.2-11.9	16.0±4.4 a	9.4-24.6	20.3±4.0 a	12.9-27.1
Medium-grain	6.1±0.3	5.6-6.5	9.7±0.9 a	8.3-11.5	19.7±7.9 a	12.3-44.6	22.8±3.8 b	15.8-28.6
Long-grain	7.0±0.3	6.7-7.4	9.9±1.4 a	7.1-11.7	19.2±7.2 a	9.5-40.0	24.6±3.4 c	17.1-31.3

Table 4 also demonstrates the wide variation of iron content in Chinese rice. It appears that the variability in the short-grain rice is slightly lower than in the other groups.

The mean value of zinc levels in brown rice of different kernel lengths (table 4) reveals average zinc contents of short-, medium- and long-grain rice to be 20.3, 22.8, and 24.6 mg kg^{-1}, respectively. The mean levels differ significantly ($p<0.05$). The zinc content is also correlated with kernel length and L/W ratio ($p<0.01$).

Effect of growing regions on the content of phytic acid, iron and zinc

It may be expected that the growing environment (Phuong et al. 1999), location and agricultural practice will influence mineral levels in rice. There are six rice growing regions in China with different natural conditions and agrotechnological infrastructure. Table 5 presents some characteristics on soil types, rainfall, temperature and sunshine of the growing regions. Table 6 shows the differences of phytic acid, iron and zinc levels in rice from different growing regions.

Table 5: Natural conditions in major rice growing regions of China

Growing regions [a]	Major Soil types	Average rainfall in growing period (mm)	Average temperature (cumulative temperature) (°C)	Cumulative sunshine in growing seasons (h)
A	Lateritic red soil, red earths, humid-thermo ferralitic soils	700-1200	⩾ 10 (5800-9300)	1000-1800
B	Lateritic red soil, yellow-cinnamon soils	700-1600	⩾ 10 (4500-6500)	700-1500
C [b]	Yellow earth, yellow-brown earths, purplish soils, torrid red soils	500-1400	⩾ 10 (2900-8000)	800-1500
D	Solonetzs, lime concretion black soils	580-1000	⩾ 10 (4000-5000)	2000-3000
E	Chernozems, meadow soils	350-1100	⩾ 10 (2000-3700)	2200-3100
F	Grey desert soils, meadow soils, cumulated irrigated soils	50-600	⩾ 10 (2000-4250)	2500-3400

[a]: see figure.1 for explanation of growing regions; [b]: altitude of this region is 160-2700 meters, the other regions are lower altitudes

If all samples are taken into account, the growing regions did not have a significant effect ($p <0.05$) on the phytic acid and iron levels. However, when comparing locations pair-wise by *t*-test, we could distinguish ($p <0.05$) groups with low, medium, and higher phytic acid content. Particularly, the special varieties have higher phytic acid, and rice grown in Jilin significantly lower phytic acid levels. The level of iron was significantly ($p <0.05$) higher in the rice cultivated in Jiangxi, and lower in Fujian, Jiangsu and Jilin. Zinc content in the rice from different growing regions was significantly different ($p < 0.05$). In particular, the special varieties had higher, and rice cultivated in Jilin, Guizhou and Ningxia had lower zinc levels than elsewhere.

Table 6: Phytic acid (PA), iron (Fe) and zinc (Zn) content in brown rice according to growing regions (mean ± standard deviation)

Growing region	Locations	Varieties (n)	PA (g kg^{-1})*	Fe (mg kg^{-1}) *	Zn (mg kg^{-1})*
A	Guangxi	6	9.2 ± 0.6a	19.6 ± 12.3a	22.6 ±1.1a
B	Anhui	9	9.4 ± 1.8a	17.9 ± 6.1a	21.9 ± 5.2a
B	Fujian	3	10.8 ± 0.5b	14.2 ± 0.1b	24.3 ± 2.4a
B	Hunan	6	10.2 ± 0.8b	18.3 ± 2.4a	26.1 ± 2.7a
B	Jiangsu	5	9.5 ± 0.8a	14.7 ± 2.7b	22.6 ± 3.1a
B	Jiangxi	5	9.2 ± 1.7a	25.2 ± 8.9c	25.7 ± 3.6a
C	Guizhou	4	9.6 ± 1.3b	19.3 ± 5.4a	18.6 ± 2.6b
D	Special varieties	3	11.24 ± 0.8c	16.5 ± 2.2a	28.1 ± 1.7c
E	Heilongjiang	5	9.9 ± 0.4b	19.9 ± 6.4a	22.6 ± 4.6a
E	Jilin	5	8.5 ± 0.6d	14.5 ± 6.2b	17.7 ± 1.9b
F	Ningxia	5	9.2 ± 0.5a	17.2 ± 3.2a	19.7 ± 2.4b
	Total/Mean	56	9.58 ±1.2b	18.13 ± 6.6a	22.55 ± 4.2a

*: different characters in same column indicate significant differences ($p < 0.05$)

The mean of phytic acid content of aerobic rice varieties, cultivated in Anhui, was 7.93 g kg^{-1} and differed significantly ($p < 0.05$) from those of irrigated varieties (11.09 g kg^{-1}) in the same location. Mean values of iron and zinc in aerobic rice were 17.15 and 19.46 mg kg^{-1}, and 22.05 and 25.69 mg kg^{-1}, respectively for irrigated varieties which were not statistically significant.

DISCUSSION

We can conclude that the levels of iron, zinc and phytic acid in Chinese varieties are very diverse. This can be explained by the genetic characteristics of varieties (Zimmermann and Hurrell 2002), the different soil and climatic properties as were shown in table 5, agricultural practice (such as irrigation and fertilization), post-harvest conditions and handling (Kennedy et al. 2002; Phuong et al. 1999). Cultivation practice and variety both influence the seed morphology, and may also affect the levels of minerals mostly occurring in chelated form with phytic acid (Otegui et al. 2002). This would suggest that there is scope for improvement of mineral content by selecting optimum varieties for specific regions. The big effect of agricultural practice is exemplified by the two groups of Australian rice that had been grown under different conditions: group A had been collected from farmers (Marr et al. 1995), while group B had been cultivated in experimental plots (Villareal et al. 1991). Chinese rice cultivation conditions are diverse: Of the six rice growing regions (figure 1), regions A and B are dual-planting areas, region C is a dual-harvesting area, and the other three are single season harvesting areas. Except for differences in planting and harvesting times, irrigation systems in these regions are also different. These differences may be expected to result in different properties of the seeds (Zhou 1993). Because of the variations of soil and climatic conditions within growing regions, no firm conclusion may be drawn yet concerning the regional effect on phytic acid levels. However, it appears that iron contents are related to soil types, considering the different iron levels in rice from region B. More agronomical research will be required to link environmental and agrotechnological factors to processing characteristics of rice and its macro- and micronutrients.

NUTRITIONAL ASPECTS

The intake of iron and zinc from rice will contribute about 12-75% and 20-81% of RDI's (Recommended Dietary Intakes) for adult Chinese if we calculate on the basis of the recommendation of the Chinese Nutrition Society (Chinese Nutrition Society 2003). Regarding these percentages, three important aspects should be noted. First, the percentages of RDI were based on the consumption of brown rice, although Chinese people seldom consume brown rice in daily life. Second, an important amount (54-66%) of minerals is lost as a result of milling and polishing (Villareal et al. 1991). This indicates that the estimated contribution to RDI is over-optimistic and that although some rice varieties could provide most of RDI's of iron and zinc for Chinese, there actually still is a big problem of iron and zinc deficiency. Third, bioavailability of iron and zinc in brown rice is much lower than recommended for the diet (Chinese Nutrition Society 2003). This implies that there is a need to minimize mineral losses during milling and polishing and to maximize the bioavailability of minerals by dephytinization.

CONCLUSION

From this study we conclude that the levels of iron and zinc in Chinese rice are very diverse.

This is both due to varietal and environmental effects. In principle, brown rice potentially will provide adequate intake of iron and zinc. However, the bioavailability of iron and zinc is very low because of the presence of phytic acid, even in rice varieties with the lowest phytic acid levels and highest levels of iron and zinc. With white rice, the situation is even worse, since 70-80% of minerals may be lost with the bran during milling.

Our study suggests that improving bioavailability by optimizing the combination of variety and growing conditions in terms of high iron and zinc and low phytic acid, will only have limited effect. Such procedures will have to be combined with post-harvest processing methods that retain essential minerals on the one hand and increase their bioavailability.

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Chapter 3

Milling characteristics and distribution of phytic acid and zinc in long-, medium- and short-grain rice

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Abstract

Milling and polishing are important operations during the production of white rice. The degree of milling and polishing has a significant effect on the nutritional aspects of white rice, especially on minerals, due to a non-uniform distribution of nutrients in the kernel. Information on the distribution of nutrients in rice will greatly help to understand the effect of milling and aid in designing procedures that improve technological and sensory properties of rice while retaining its essential nutrients as much as possible. In this study, three kernel shapes (short-, medium- and long-grain) of rice were selected for the study of milling characteristics and distribution of zinc and phytic acid using abrasive milling and X-ray fluorescent microscope imaging approaches.

Milling characteristics differed with kernel shapes and varieties. Mass loss (y, %) correlated well with milling duration (x, s) and was fitted using a polynomial equation of $y = ax^2+bx+c$ ($R^2= 0.99$). Different kernel shapes of rice resulted in different patterns. Breakage in milling increased with longer duration of milling. The relation between breakage (y, %) and milling duration (x, s) fitted the exponential equation $y = ae^{bx}$. Levels of phytic acid, as well as zinc decreased with prolonged milling. Phytic acid decreased at a higher rate than zinc. The analysis of different milling runs showed that the concentration of phytic acid decreased from the surface region inward, whereas X-ray fluorescent images indicated that the highest concentration of phosphorus was at the interface of embryo and perisperm.

Our results help to understand the milling characteristics of different rice varieties. Understanding these characteristics offers opportunities to optimize milling procedures for maximum phytate removal, at minimum mineral losses and yield loss.

INTRODUCTION

Rice is one of the important cereals in the world. It is commonly used as milled or white rice produced by removing the hull and bran layer of the rough rice kernel (paddy) in hulling and milling processes, respectively (Perdon et al. 2001). Brown rice (hulled or dehulled rice) is composed of surface bran (6-7% by weight), endosperm (≈90%) and embryo (2-3%) (Chen et al. 1998). White rice is referred to as polished or whitened rice when 8-10% of mass (mainly bran) has been removed from brown rice (Kennedy et al. 2002). Milling by abrasion is an important mechanical procedure for white rice production. During milling, brown rice is subjected to abrasive or friction pressure to remove bran layers resulting in high, medium or low degrees of milling depending on the yield of head rice and the shape of the kernels (Chen and Siebenmorgen 1997; Chen et al. 1998). Milling brings about considerable losses of nutrients and affects the edible properties of milled rice (Chen et al. 1998; Doesthale et al. 1979). As most cereals, rice does not show a homogeneous structure from its outer (surface) to inner (central) portions (Itani et al. 2002). As a consequence, information on the distribution of nutrients will greatly help to understand the effect of milling and aid in improving sensory properties of rice while retaining its essential nutrients as much as possible.

Depending on the extent of milling, changes of some nutrients, such as surface lipids (Chen et al. 1998; Perdon et al. 2001), protein (Chen et al. 1998; Heinemann et al. 2005), physical properties such as rice paste viscosity (Perdon et al. 2001), and sensory quality of milled rice, including taste (Park et al. 2001; Tran et al. 2004), have been reported. Effect of milling on some macro- and micro-elements, e.g. iron, magnesium, phosphorus, phytic acid, have also been studied. Early studies described the effect of milling on minerals or distribution of minerals according to approximate milling degrees, such as lightly milled, reasonably milled and well milled, or as fractions I, II and III, respectively (Kennedy and Schelstraete 1975; Song et al. 1988; Tabekhia and Luh 1979). These authors did not provide detailed information about the distribution of these nutrients in rice kernels. Itani et al. reported the distribution of some nutrients in more detail, although phytic acid and trace minerals were not included (Itani et al. 2002). Studies on Indian rice indicated that the extent of milling had a significant effect on losses of magnesium and calcium, but not on phosphorus and trace minerals ($p<0.05$) (Bajaj et al. 1989). Recently, X-ray fluorescent microscopy techniques were developed and applied to map the distribution of minerals such as magnesium, potassium, phosphorus, calcium and sulphur in quinoa seeds (Emoto et al. 2004; Konishi et al. 2004).

Considering world wide deficiencies of iron and zinc, our ultimate aim is to improve the bioavailability of these minerals by reducing insoluble mineral-phytate complexes, and fortification if desired. As the zinc-phytate complex is very stable (Vasca et al. 2002), we focussed on zinc as a target mineral. Our previous study indicated that zinc and phytate contents in Chinese rice varieties cover a broad range (Liang et al. 2007). The purpose of the present study was to compare the milling characteristics and the distribution of zinc and phytic acid in long-, medium- and short-grain kernels of rice from China, with a view to optimize for maximum phytate removal at minimum losses of zinc. Precision abrasive milling was used to obtain a range of milling degrees, and X-ray imaging methods to map the distribution of different minerals. The degree of milling for specific rice cultivars could be optimized for maximum removal of phytic acid, maximum retention of zinc, and appropriate whiteness to satisfy consumer expectations for white rice.

METHODOLOGY

Paddy rice and characteristics

Based on our survey of the variation of phytic acid and minerals in rice varieties cultivated in China (Liang et al. 2007), three varieties namely Ganwanxian 30 (long-grain, G30), Zhongyou 752 (medium-grain, Z752) and Bijing 37 (short-grain, B37) having different levels of phytic acid and minerals were selected. G30 and Z752 were obtained from the Jiangxi Seeds Company, and B37 from the Academy of Agricultural Science of Guizhou. All paddies were harvested during the autumn of 2003 and were stored dry and cool (~15°C) less than 90 days before processing and analysis. General characteristics, including crude protein content (PC), yield of brown rice (YBR), breakage from hulling, shape i.e. ratio of length to width (RLW), kernel surface area (KSA), and thousand-kernel weight (TKW), are presented in table 1. According to the industrial standard of China, the three cultivars were classified as long-grain, medium-grain and short-grain, respectively (CHISA 2002).

Hulling and Milling

Paddy was dehusked with a lab-scale hulling machine (THU-35C, Satake, Japan). Each variety was assessed in triplicates for thousand-kernel weight (TKW), yield of brown rice (YBR) and breakage from hulling.

Only unbroken brown rice kernels were used for subsequent milling experiments. About 30 (± 1) g of brown rice were milled for the duration of 6, 10, 20, 30, 45, 60, 90, 180 and 300 s, respectively, with a lab-scale milling machine (TM 05C, Satake, Japan) to obtain rice milled to different extents. Each milling treatment (duration) was performed in triplicates. Yields of white rice and breakage from milling were measured. After milling, whole milled rice kernels (head rice) were separated, and then ground with grinder (HY-04B, Beijing Xinhuanya, China) to pass a 1 mm sieve, and dried at 100°C till constant weight. Dried rice flour was kept in sealed plastic bags at 4°C until chemical analysis.

Table 1: General characteristics of brown rice samples

Variety	Collection	PC (g $100g^{-1}$)[a]	Length (mm)	RLW[b]	KSA (mm^2)[c]	TKW (g)[d]	Yield of brown rice (%)[e]	Breakage (%)[f]
Bijing 37 (Yu et al. 2001)	Guizhou	8.8	5.2	1.8	68	25.5	80.6	3.7
Zhongyou 752 (Centre of China Crop Science and Technology 2006)	Jiangxi	10.9	6.4	2.8	50	26.5	71.9	16.2
Gangwanxian30 (Information of Jiangxi foodstuff-oil and soil-fertilizer 2006)	Jiangxi	8.5	7.6	3.4	52	27.6	73.6	28.0

[a]: PC: protein contents (provided by supplier, see reference)

[b]: RLW: ratios of length to width (analysed in this study)

[c]: KSA: kernel surface area, assumed that the shape of integral kernels were separated into two semi-sphere and a cylinder. Surface area was calculated with $4 \times (\text{width}/2)^2 \times \pi + 0.9 \times \pi \times \text{width} \times \text{length}$ (analysed in this study)

[d]: TKW: thousand-kernel weight (provided by supplier, see reference)

[e]: On wet mass basis. Unless mentioned, percentage in this paper was calculated on wet weight mass basis (analysed in this study)

[f]: Breakage caused by dehulling: percentage of broken brown rice weight to total brown rice weight (analysed in this study)

Zinc

Samples of 0.5 g (accuracy 0.1 mg) dried rice flour were digested using a microwave laboratory system (Milestone, Italy) with nitric acid (HNO_3, reagent grade) and hydrogen peroxide (H_2O_2, analytical reagent, Beijing Chemical Works, China) as described by D'Ilio et al. (2002). Contents of zinc in solutions were measured with a Vario 6 Atomic Absorption System (Analytik Jena, Germany). Each sample was digested and measured in triplicates.

Phytic acid

Phytic acid levels in brown rice and milled rice were analysed using a modified AOAC method as described by Ma et al. (2005). All materials were analysed in triplicates.

X-ray fluorescent imaging

X-ray elemental maps were obtained with a micro-X-ray fluorescence instrument developed at Osaka City University (Emoto et al. 2004). The X-ray tube (MCBM 50-0.6B, rtw, Germany) with Mo target was operated at 50 kV and 0.45 mA. The tube was installed into an X-ray tube shield holder equipped with an X-Y-Z positional device, where the polycapillary X-ray lens was attached. The polycapillary lens was designed and manufactured at Beijing Normal University. The length, input focal distance, and output focal distance were designed to be 50 mm, 34 mm, and 16 mm, respectively. A spot size of about 40 mm was obtained at a focal point. A silicon drift X-ray detector (SDD, X-Flash Detector, Type 1201, Rontec, Germany; sensitive area: 10 mm^2, energy resolution: <150 eV at 5.9 keV) was suspended using a down-looking geometry. The sample stage was placed on the X-Y-Z stage [YA05A-R1 (X-Y stage) and ZA07AR3S (Z stage); Kohzu precision, Japan], which was controlled by stepping motors driven by a computer. To control the sample stage, motor drivers and a motor controller (NT2400, Laboratory Equipment Co., Japan) were applied. An SDD signal was analysed by a multi-channel analyser (NT2400/MCA, Laboratory Equipment Co., Japan). To confirm the position of the sample, a visible CCD camera was also installed.

RESULTS AND DISCUSSION

Milling characteristics

For the miller, the main quality characteristic of rice is related to the amount of material that needs to be removed to obtain white rice. During the abrasion of rice, not only the outer layers of the kernels are removed, but also kernels are broken. These are also considered a loss. We therefore defined milling characteristics as the mass loss and breakage during milling. The results for the rice cultivars used in this study are shown in figure 1a and 1b.

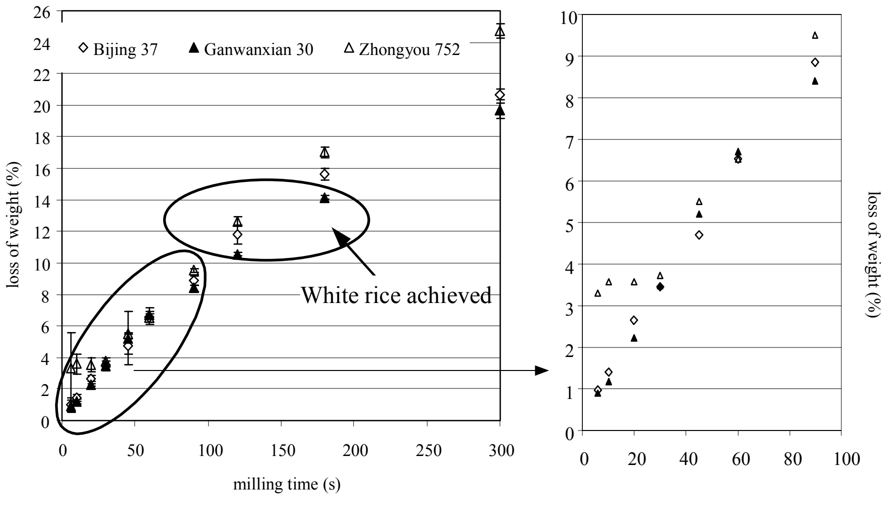


Figure 1a: Mass loss during milling

Figures 1a and 1b show the loss of mass, and breakage, with increasing milling time. For the three varieties tested, mass losses (figure 1a) were similar: with increasing milling duration more of the outer layers is removed. Loss rates become less at longer milling times.

The relation of mass loss (y, %) vs duration of milling (x, s) fitted a polynomial equation of $y = ax^2+bx+c$ ($R^2=0.99$), as was observed in other studies (Perdon et al. 2001; Singh Gujral et al. 2002). Different kernel shape of rice resulted in different patterns. Z752 had a higher mass loss (figure 1a) at each milling time. To achieve a mass loss of 2%, it took less than 10 s for Z752, and about 20 s for B37 and G30, respectively. Generally, in order to obtain white rice, about 10-15% of mass is removed from the outer layers. In this study, Z752 required the shortest duration of milling to obtain white rice, followed by B37 and G30. The differences among mass loss patterns of the three cultivars might be related to the removal of the embryo. Visual inspection to check the removal of the embryo from brown rice during milling revealed a large variation between the different rice varieties. Under our experimental milling conditions, one third of the kernels of Z752 and B37 had lost their embryo after 30-45 s milling, corresponding to a mass loss of 3.5-4.5%, whereas more than 90% of the kernels had lost their embryo after 90 s milling, at mass loss of 8.5-9.5%. For G30, about 30% of kernels had lost their embryo at 2% mass loss, whereas after 45 s milling, most kernels had lost their embryo at a mass loss of 5%.

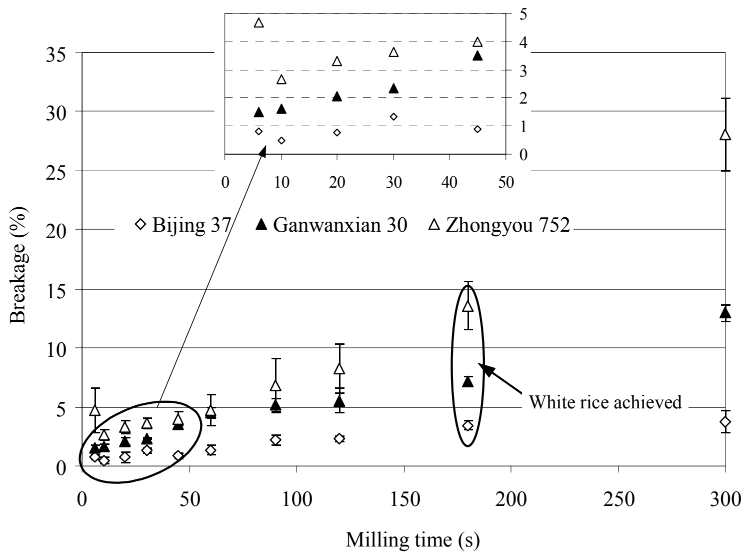


Figure 1b: Breakage during milling

Rice breakage was quantified as the weight of broken rice expressed as a percentage of the total weight of milled rice (Chen et al. 1998). Breakage of the three cultivars was similar (figure 1b), increasing with longer duration of milling. The relation between breakage (y, %) and milling duration (x, s) fitted the exponential equation $y = ae^{bx}$. The different cultivars had different a and b values, while R^2 ranged from 0.76 to 0.95. Of the three cultivars, Z752 had the highest breakage values, and B37 had the lowest at each time interval. After 90 s milling, breakage of Z752 had reached 30%, while breakage of B37 was still low (about 5%). The amount of broken kernels of long- and medium-grain in the present study were of a similar magnitude as of long-grain rice in a single-break commercial milling system (Chen et al. 1998).

Phytic acid and zinc levels

Phytic acid and zinc levels in rice after increasing extent of milling are presented in table 2.

Table 2: Contents of phytic acid (PA) and zinc (Zn) in milled rice*

Milling time (s)	**Bijing 37**		**Zhongyou 752**		**Ganwanxian 30**	
	PA* (g kg^{-1})	**Zn (mg kg^{-1})**	**PA (g kg^{-1})**	**Zn (mg kg^{-1})**	**PA (g kg^{-1})**	**Zn (mg kg^{-1})**
0	8.9 ± 0.1	22.1 ± 0.7	7.8 ± 0.1	22.8 ± 1.0	11.1 ± 0.1	19.3 ± 4.9
6	9.0 ± 0.3	20.8 ± 2.5	7.6 ± 0.1	20.7 ± 0.1	8.1 ± 0.3	20.3 ± 4.8
10	8.9 ± 0.2	20.0 ± 0.6	7.9 ± 0.2	22.6 ± 0.3	7.5 ± 0.3	20.0 ± 3.6
20	6.9 ± 0.3	19.8 ± 0.5	8.7 ± 0.3	22.6 ± 0.8	7.5 ± 0.4	19.0 ± 2.9
30	6.5 ± 0.2	21.7 ± 1.3	6.8 ± 0.2	21.5 ± 1.2	8.0 ± 0.3	22.7 ± 1.4
45	6.3 ± 0.2	22.3 ± 2.6	6.5 ± 0.3	18.1 ± 0.8	5.9 ± 0.6	21.9 ± 0.7
60	4.8 ± 0.1	21.2 ± 1.3	6.3 ± 0.5	17.7 ± 0.9	5.3 ± 0.6	21.3 ± 2.2
90	3.1 ± 0.1	20.1 ± 1.8	4.7 ± 0.2	16.3 ± 0.2	4.9 ± 0.2	25.8 ± 4.0
120	2.1 ± 0.1	15.0 ± 1.0	3.2 ± 0.4	16.4 ± 0.2	3.3 ± 0.1	20.5 ± 1.1
180	0.7 ± 0.0	14.8 ± 0.7	0.9 ± 0.0	15.9 ± 0.3	1.2 ± 0.1	21.2 ± 0.3
300	0.2 ± 0.0	13.7 ± 1.7	0.1 ± 0.0	16.7 ± 1.1	0.2 ± 0.1	18.5 ± 2.8

* All data are based on dry mass weight and are presented as average ± standard deviations (n=3)

Levels of phytic acid and zinc decreased with prolonged milling. Although it has been observed earlier (Itani et al. 2002) that all minerals (including phosphorus) decrease from the outermost fraction, it appears here that phytic acid levels decrease at a higher rate than those of zinc.

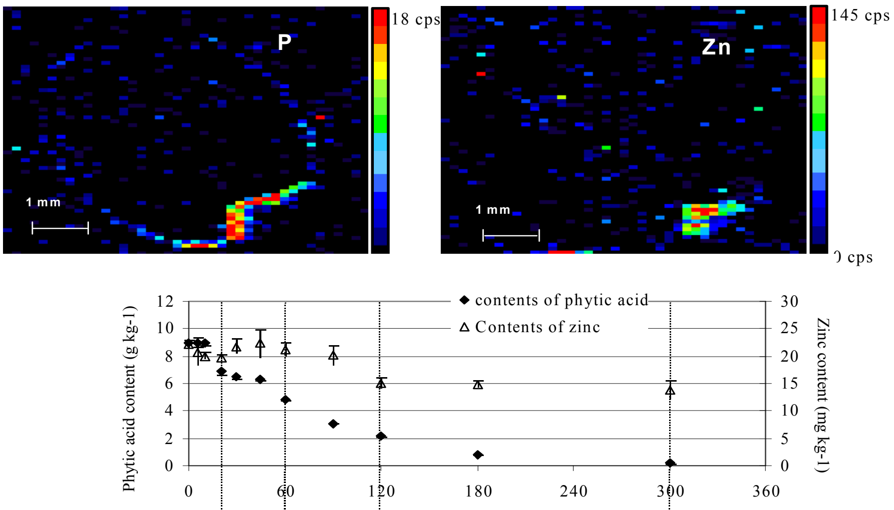
Phytic acid levels in brown rice of B37, Z752 and G30 were 8.9, 7.8 and 11.1 g kg^{-1}, respectively. These values differ from our previous results (Liang et al. 2007) probably due to differences of cultivating environments and agricultural practice (Liu et al. 2005a). G30 had the highest phytic acid content in raw brown rice and this decreased quickly during milling. After 30 s milling, phytic acid levels in milled rice of B37 and Z752 were at the same level (7 g kg^{-1}) although they started at different initial values, and it was 8 g kg^{-1} in G30 after the same milling time. After 120 s milling (duration considered to be optimum in commercial milling of white rice), phytic acid in G30 and Z752 were still at the level of 3.2 g kg^{-1}, higher than in B37 (2.0 g kg^{-1}). After 300 s milling, phytic acid in all varieties were at the level of 0.2 g kg^{-1}. Phytic acid levels in brown rice decreased at a similar rate as reported elsewhere (Doesthale et al. 1979) for phosphorus.

The zinc levels in the brown rice varieties studies were similar (22.1, 22.8 and 19.3 mg kg^{-1}, respectively), and even after 30 s milling (corresponding to a mass loss of about 5%), the zinc levels in all varieties of milled rice were still at the same level of brown rice. The biggest lost of zinc in Z752 occurred after 45 s milling, and in B37 after 120 s milling.

However, in G30, up to 120 s milling did not affect its zinc level, a phenomenon that has been previously reported elsewhere (Juliano 1972; Villareal et al. 1991). With some milled rice samples even higher zinc levels were reported than in the initial brown rice (Heinemann et al. 2005). In the three varieties studied here, zinc levels after 300 s milling were 4-38% lower than that of initial values.

Location and distribution of phytic acid and zinc in brown rice

In order to visualize the distribution of phytic acid and zinc in brown rice, X-ray fluorescent microscope imaging techniques were used. Images of location of phytic acid (indicated as phosphorus, P) and zinc obtained with X-ray fluorescent scanning, as well as the distribution of phytic acid and zinc in brown rice kernels obtained by abrasive milling are shown in figures 2a, 2b and 2c and demonstrate the location of P and zinc (Zn) in rice kernels.



Situation of kernel	Brown rice		Milled rice	Core of kernel
Milling time (s)	20	60	120	300
Mass loss (%)	2.7	6.5	11.8	20.7
Loss of PA (%)	23	46	76	98
Loss of zinc (%)	13	10	40	51
Molar ratio of PA/Zn	23	15	9	<1
Regression of PA (y, %) retention to milling time (x, s)	$y = -0.0322\,x + 7.7374$	$R^2 = 0.8236$		

Figure 2a: Distribution of phytic acid and zinc in Bijing 37 (short-grain)

In all three varieties, the density of phosphorus decreased from the surface region inward. This agrees with data from abrasive milling experiments (Bryant et al. 2005). The embryo region did not contain much phosphorus, whereas the highest density was observed at the interface of embryo and perisperm. Phosphorus may be located at different positions in different rice varieties. In B37 (figure 2a) and G30 (figure 2c), its distribution was similar; there were distinct regions at the outermost surface layer where phosphorus was concentrated.

But in Z752 (figure 2b) the distribution differed from the other varieties, having no distinct layer of higher phosphorus concentration. Notably, at the side of the embryo, we could not observe the high density of phosphorus as observed in figures 2a and 2c. The distribution of phosphorus in the rice kernels suggested that at least the outer layer should be removed if we want to significantly decrease phytic acid in milled rice, since 70-85% of phosphorus occurs as phytic acid in rice. The location of zinc in the three varieties was similar. All three varieties had the highest density of zinc in the embryo whereas zinc was relatively evenly distributed in the other regions. This helps us to understand earlier reports, that milling degrees higher than 10% had little effect on zinc levels in milled rice (Bryant et al. 2005). The location of zinc indicates that it may be beneficial to retain more embryo to obtain higher final levels of zinc.

We observed an inverse relation: $y = a-bx$ ($a=7.7-8.3$, $b=0.03$, $R^2=0.82-0.91$) between phytic acid levels (y, mg g^{-1}) and milling duration (x, s) (figure 2a, 2b, 2c). This relation shows some similarity with that between milling degree and surface lipid, and phenolic acids observed elsewhere (Perdon et al. 2001; Zhou 2003). In B37 and Z752, about 23 to 33% of total phytic acid was located in the surface outer layer of kernel (2-3% weight percent of brown rice). In all varieties, about 23-25% of total phytic acid was located in the sub-surface layer, which accounted for 3.4-4.5% of total weight. Less than 2% of total phytic acid was located in the 75-80% of central portion of kernel. The remaining 40-50% of phytic acid was located in the mesocarp of brown rice, representing 13-15% of kernel weight. The distribution of phytic acid observed from milling experiments is very well supported by images obtained from X-ray scanning. Differences in the distribution of phytic acid location in varieties mainly occurred in the outermost layer. In this region, distribution of phytic acid in B37 and Z752 was similar, and relatively even at the outermost surface with a steep decrease inward. However, the distribution in G30 was quite different, showing a steep decrease already at the outermost surface layer, followed by a relatively even distribution. This distribution pattern was similar to the distribution of phosphorus in other rice varieties (Bajaj et al. 1989). The perisperm is another layer of the kernel, removed at the interval of milling duration from 60 s to 120 s. Phytic acid located here varied by 20-40%, with weight percent at 4-6% in different cultivars.

The distribution of zinc in the three varieties was different. For B37, distribution of zinc was relatively even in the layer occupied 30% of the total kernel weight, with a steep decrease in next region, and followed with another even distribution in the central part. In contrast, in Z752, a steep decrease of phytic acid occurred at sub-surface layer, at the milling interval from 30 to 60 s (occupied about 4% of total weight of kernel), and with an even distribution in other parts. For G30, the distribution of zinc was relatively even from the surface to the central part of the kernel. The highest decrease occurred at the interval of 60-120 s. Further analysis of regression showed there was no correlation between zinc contents and milling duration. Figures 2 also showed that more than 60-70% of total phytic acid was located in the 10% of surface layer, and less than 40% of zinc was in the same layer. The different distribution patterns of phosphorus and zinc should enable an optimized milling, removing as much as possible phytate while retaining relevant levels (at least 50%) of zinc.

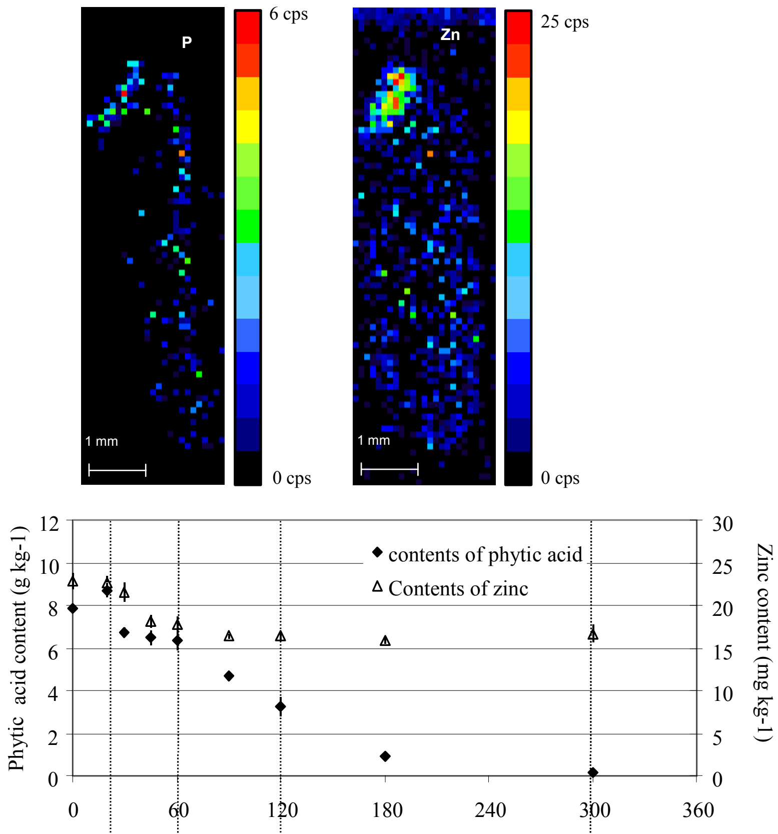
Molar ratios of phytic acid to zinc varied with regions in kernel and cultivars. For all varieties, only when more than 20% of outer layer was removed, molar ratios of phytic acid to zinc could decrease to less than 1. This was achieved only after 300 s milling, which is considerably longer than the standard commercial practice.

DISCUSSION

Milling consequences, such as mass loss and breakage, could be affected by intrinsic factors (e.g., variety, kernel shape) as well as extrinsic factors (e.g., milling equipment). Under identical processing conditions, rice can display different processing properties. These can be caused by e.g. variety, maturity, and cultivating conditions, and can influence mass loss and breakage because of different shape, hardness of kernels, and thickness of the aleurone layer (Juliano 1972; Zhou 2003). Different bran loss rates by milling have been attributed to shape and hardness of grains, as well as pericarp thickness, oil bodies, cellulose, and hemicelluloses in bran layers (Juliano 1972; Mohapatra and Bal 2004; Singh Gujral et al. 2002; Singh et al. 2000). These would contribute to the differences observed for the three varieties and the higher mass loss rate after short milling periods. Cultivars differ in thickness of the aleurone layer and in hardness distribution in the endosperm. *Japonica* (bold or coarse short-grain) kernels tend to have more cell layers than *indica* (slender long- or medium- grain) kernels. The central core and the mesocarp in *indica* and *japonica*, respectively are hard, and kernel hardness is negatively correlated to length-to-breadth ratio (Juliano 1972). Combined effects of such factors may have caused the relatively high mass loss of Z752 from milling.

In addition to the effect of equipment and process conditions, the composition, structure and thickness of rice kernels also affect the extent of breakage from milling (Siebenmorgen and Qin 2005; Zhou 2003). Whereas breakage could not be related to kernel width or length (Siebenmorgen and Qin 2005), the susceptibility to relative humidity and fissuring could play a role in breakage (Lloyd and Siebenmorgen 1999). Further investigation is required to help understand the mechanism for the high extent of breakage from milling in Z752.

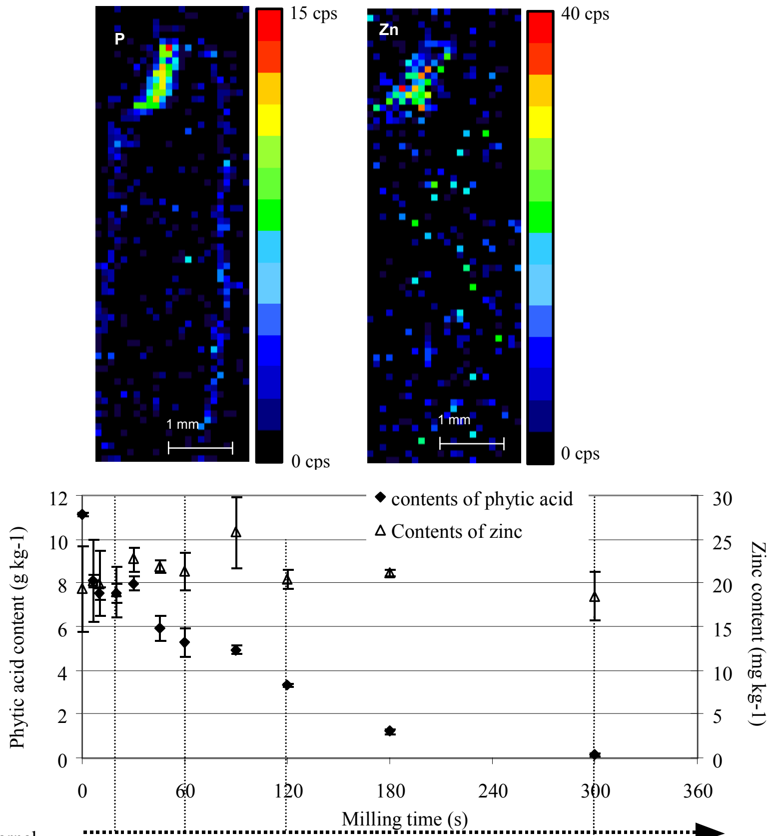
Phytic acid is an important storage of phosphorus and minerals present in seeds. It usually occurs as a mixed salt of potassium and magnesium, and may also contain calcium, zinc and/or iron. Phytate has a different accumulation pattern from protein reserves which are mainly deposited within the numerous protein bodies in seed storage cells (Liu et al. 2004; Liu et al. 2005b). Studies on *japonica* rice indicated that phytic acid levels were not related to protein levels, which were significantly influenced by genetic and environmental factors (Juliano 1972; Liu et al. 2005a; Liu et al. 2005b). Our milling experiment indicated that about 25% of the phytic acid is located in the perisperm of the kernel, which differs from earlier findings that phytic acid was only present in the aleurone layer after embryo removal (Liu et al. 2004). This difference suggests that although phytic acid is approximately located in the outer layer of kernel, the precise distribution might differ among rice varieties. In order to obtain milled rice with minimum weight losses but maximum removal of phytic acid, the distribution of phytic acid in the kernel should be established first, and this should form the



Situation of kernel	Brown rice		Milled rice	Core of kernel
Milling time (s)	20	60	120	300
Mass loss (%)	3.1	6.5	12.6	24.7
Loss of PA (%)	---	25	64	99
Loss of zinc (%)	4	27	37	45
Molar ratio of PA/Zn	38	35	20	<1
Regression of PA (y, %) retention to milling time (x, s)	y = -0.0322 x + 8.2556 R^2 = 0.8456			

Figure 2b: Distribution of phytic acid and zinc in Zhongyou 752 (medium-grain)

basis to determine the appropriate milling treatment. Our X-ray microscope images indicate that in absolute terms, very little phosphorus is located in the embryo itself, which differs from the observation made earlier (Liu et al. 2004) that phosphorus concentration in embryos was about 5 times higher than in whole kernels. Both abrasive milling experiments and X-ray images indicate that zinc was not mainly located in rice bran, unlike total ash or other minerals (Dikeman et al. 1980; Doesthale et al. 1979; Kennedy et al. 2002; Resurreccion et al. 1979). From our X-ray images, it can be observed that the embryo has the highest concentration of zinc. It however represents a very small fraction of the total grain and in absolute terms, does not contribute very much to the total zinc in milled rice.



Situation of kernel	Brown rice		Milled rice	Core of kernel
Milling time (s)	20	60	120	300
Mass loss (%)	2.2	6.7	10.5	19.7
Loss of PA (%)	33	52	70	99
Loss of zinc (%)	4	---	6	23
Molar ratio of PA/Zn	39	24	16	<1
Regression of PA (y, %) retention to milling time (x, s)	$y = -0.0304x + 7.907$ $R^2 = 0.9106$			

Figure 2c: Distribution of phytic acid and zinc in Ganwanxian 30 (long-grain)

The different distributions of phytic acid and zinc in rice kernels confirmed earlier statements that phytic acid is primarily present in the potassium or magnesium form instead of the zinc form (Dikeman et al. 1980). Further research is required to assess the effects of other factors such as environmental conditions and agricultural practice, on the distribution of phytic acid and minerals in rice.

CONCLUSION

From this study we conclude that milling characteristics, including mass loss and breakage, varied with rice kernel shape and/or rice varieties. This indicates an opportunity for optimized milling, dedicated to improve the quality of white rice.

The distribution of phytic acid differs in rice varieties, as well as in parts of rice kernel. Most of the phytic acid in rice kernels is located in the outermost layer. Zinc distribution is similar in different rice varieties, and relatively even in rice kernels, except for the high density in embryo. The results give us the possibility to process brown rice to obtain low phytic acid contents at a relatively high zinc content.

ACKNOWLEDGEMENT

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Chapter 4

Effect of soaking and phytase on phytic acid, calcium, iron and zinc in rice fractions

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(submitted)

Abstract

Rice is an important dietary source of minerals for people in developing countries. Uptake of minerals from different rice products depends on their concentration as well as their availability, both being affected by food processing. In the present study, the effects of wet processing (pre-heating, soaking 1 week in demineralised water, acidic buffer, and 30 min in phytase solution) of rice fractions (white rice kernels and flour, brown rice kernels and flour, and bran) on levels of minerals and phosphorus were studied.

A dry heat treatment was desired to distinguish possible effects of endogenous phytases confounding those of added phytase. White rice contained 4 mg g^{-1} phytic acid which was removed by all treatments to below detection levels. Dry matter losses due to soaking ranged from 2-20% with highest losses observed after soaking in acidic buffer. Whereas relatively high losses of calcium (12-63%) and iron (9-43%) were recorded, zinc losses were lower (0-38%).

Brown rice contained 20 mg g^{-1} phytic acid which was removed best from kernels (75% of initial) by soaking in acidic buffer, but in flour by phytase application to below detection levels. Dry matter losses due to soaking ranged from 0.1-31% with highest losses observed after soaking in acidic buffer. Brown rice contains about two-fold higher levels of calcium, iron and zinc than white rice. However, losses due to soaking were similar as in white rice, for calcium (22-62%), iron (31-60%), and zinc (8-50%).

Bran contained 140 mg g^{-1} phytic acid which was removed best (-92%) by phytase treatment, followed by soakingin acidic buffer (-50%). Dry matter losses due to soaking were highest of all rice fractions (7-30%). Bran contains the highest levels of calcium (671 mg g^{-1}), iron (95 mg g^{-1}) and zinc (57 mg g^{-1}), and phytase treatment resulted in dissolution of 50-70% in the soaking medium. Our results indicate that phytase application is an effective method to rapidly remove of phytic acid while preserving dry matter and minerals in rice fractions.

INTRODUCTION

In China, rice plays an important role in human nutrition. It is used as staple food for about two thirds of the population. In 2002 and 2003, it was reported that intake of rice and rice products was 238 and 215 g/capita/day, respectively, representing 35% of the total energy intake (FAO 2006; Wang 2005). In the rural areas in 2002, the intake of rice and rice products was 246 g/capita/day. In addition to energy, rice also supplies important micronutrients, such as iron, zinc, calcium and some vitamins. In rural China, iron consumption was estimated at 22.4 mg/capita/day in 1992, and 23.1 mg/capita/day in 2002 (Wang 2005). Of the total iron intake, about 8.5% is estimated to originate from rice and rice products. It was reported that iron deficiency related anaemia is still prevalent, which could be associated with poor bioavailability of iron in the diet of the Chinese who nevertheless consume about 2-fold higher quantities of iron as advised in the RNI (recommended nutrients intake) (Ma 2007).

In order to improve the bioavailability of minerals in rice and rice products, we have undertaken a systematic research including (i) selection of proper varieties with higher mineral contents and lower inhibitor (phytic acid) levels (Liang et al. 2007), (ii) optimization of dry rice milling to reduce losses of minerals while removing phytic acid (Liang et al. accepted), and (iii) wet processing to achieve removal or degradation of phytic acid. Although the first two approaches resulted in some opportunities to improve bioavailability of minerals, the improvement is not sufficient to expect a significant impact. Studies on other cereals indicated that wet processes, such as soaking, germination and fermentation could effectively decrease phytic acid and improve the bioavailability of minerals. Soaking is widely applied at both household and industrial scale. Soaking could however also affect processing properties because of water absorption, and change sensory properties besides improving the nutrient availability through removal of inhibitors. Soaking is the most important operation during rice noodle making (during which a natural fermentation takes place) (Lu et al. 2005) because it softens the kernel prior to pulping. Soaking pearl millet with endogenous or exogenous phytase enzymes at optimum conditions increased the in vitro solubility of iron and zinc by 2-23% (Lestienne et al. 2005a; Lestienne et al. 2005b). Depending on the type of cereals tested, phytate in millet, maize, sorghum and rice was reduced by 4-28% (Lestienne et al. 2005d). Inhibitors in legume seeds, such as tannin, trypsin inhibitors and phytic acid, were also reduced by soaking (El-Hady and Habiba 2003; Sattar et al. 1989; Vijayakumari et al. 1998). However, simultaneously with the decrease of inhibitors, valuable nutrients may also be lost by soaking. It has been reported that soaking of soya beans (Bayram et al. 2004), common beans (Barampama and Simard 1995), and leguminous seeds (El-Adawy et al. 2000; Sattar et al. 1989) results in losses of dry matter and minerals. Despite the importance of rice for human nutrition, the opportunities and limitations of wet processing for the improvement of mineral bioavailability have not yet been sufficiently addressed.

The objectives of present study are 1) to explore the potential of soaking and phytase treatment scenarios for the removal of phytic acid from rice fractions; and 2) to identify the negative effects of nutritional relevance, i.e. losses of dry matter and minerals.

MATERIALS AND METHODS

Rice fractions

Brown rice, white rice and rice bran (derived from rice variety of Kenjian 90-31, harvested in 2005) were obtained from Beijing Huateng Model Rice Mill Company, Beijing, China. Flour fractions were prepared by grinding brown and white rice kernels using a grinder (HY-04B, Beijing Xinhuanya, China) and the product was sieved through a 1 mm screen.

Phytase

A sample of fungal phytase was obtained from DSM (Delft, the Netherlands), released only for scientific purposes. The activity is 6000U g^{-1}, the suggested dose based on application in animal feeds, was 500 U kg^{-1} dry matter (optimum temperature 55ºC, optimum pH 2.5-5.5).

Processing

Raw materials were preheated, and soaked in various soaking media as outlined in table 1.

Table1: Experimental treatments

Treatments	Methods	Code	Conditions
Preheating	Dry preheating	PD	Heat in an ventilated hot air oven at 100°C, kept for 30 min, then cool to room temperature in sealed glass vessels
	Wet preheating*	PW	Mix rice with demineralised water at 1:1 (w/v) ratio, then heat in autoclave at 115°C for 10 min, then cool and lyophilize
Soaking	Medium: Demineralised water (pH 5.95)	SDW	Mix rice (kernels or flour) with medium at ratio 1:3 (w/v) , bran with medium at 1:5 ratio (w/v), then soak in a thermostat incubator at 10°C, during 172 h (kernels) or 24 h (flour and bran)
	Medium: Acidic buffer (HAc-NaAc, mixed at the ratios 16:1, HAc (1M): NaAc (1M), pH≈3.5	SAB	Mix rice (kernels or flour) with medium at ratio 1:3 (w/v) , bran with medium at 1:5 ratio (w/v), then soak in a thermostat incubator at 10°C, during 172 h (kernels) or 24 h (flour and bran)
	Medium: Phytase solution (500 U L^{-1})	SPS	Mix rice (kernels or flour) with medium at ratio 1:3 (w/v), bran with medium at 1:5 ratio (w/v), adjust to pH5.5 with HAc and NaAc, then soak in a thermostat water bath at 50± 2°C, for 30 min

*: Because of gelatinization of starch, wet-preheated rice became sticky and impossible to separate from soaking media. Only none and dry preheated rice was therefore used for experiments of soaking and phytase treatments.

After soaking, kernels and flours were separated from liquids by decanting reaction medium directly or after centrifugation (5000 *g*, 15 min). All solid residues were freeze-dried and kept at 4ºC prior to analysis.

Phytic acid

Contents of phytic acid of all materials were analyzed according to the China National Standard Analysis Method as described previously (Liang et al. 2007).

Phosphorus in soaking media

The colorimetric method AOAC 995.11 (Horwitz 2000) was used to determine phosphorus levels. Acid soluble phosphate forms a blue complex with sodium molybdate in the presence of ascorbic acid as reducing agent. The intensity of blue colour was measured spectrophotometrically at 823 ± 1 nm (7200, Unico, Shanghai, China).

Calcium, iron and zinc

Minerals in solid residues were analysed using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 2000, Perkin-Elmer) after wet acid digestion with concentrated nitric acid (HNO_3, 65 %) and perchloric acid ($HClO_4$, 60 %) following the procedure of AOAC official method 975.03 (Horwitz 2000).

Data analysis

All treatments were carried out at least in duplicates. Data were analysed by ANOVA and *t*-tests using the SPSS package (Sony DADC, version 12.0.1).

RESULTS AND DISCUSSION

The effects of the processing treatments of rice fractions, on phosphorus (P) levels, dry matter and mineral levels are presented in figures 1-3 and table 2, respectively. Soaking led to the decrease of the concentration of phosphorus and phytic acid (PA) in solid residues and increase of phosphorus in the soaking medium.

From all three figures and table 2, the first observation is that the preheating treatment did not have significant ($p<0.05$) effects on the levels of soluble and insoluble P, and no systematic effects on dry matter and mineral contents of the rice fractions were observed.

Table 2: Effect of preheating and soaking on dry matter, calcium, iron and zinc in rice fractions

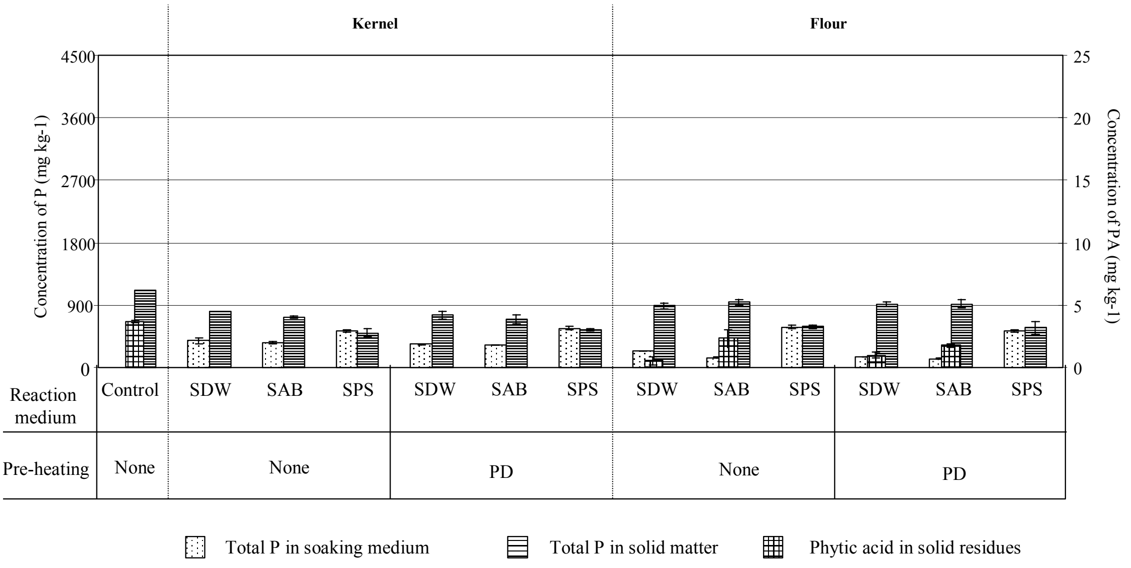
Rice fractions	Preheating code	Soaking code[1]	Dry matter loss (% of initial)	Ca (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})
White rice						
	None	Control	0	173±1	28.7±3.8	24.5±0.3
Kernel	None	SDW	8.6	127±5 (-27)	21.5±4.9 (-25)	24.5±2.6 (-0)[2]
		SAB	15.1	86±17 (-50)	18.6±3.7 (-35)	22.8±3.6 (-7)
		SPS	2.0	153±16 (-12)	25.9±3.4 (-10)	42.1±9.1 (+72)
	PD	SDW	13.0	123±23 (-29)	17.2±3.0 (-40)	23.0±3.6 (-5)
		SAB	19.7	118±10 (-32)	16.5±3.0 (-43)	24.2±3.3 (-1)
		SPS	3.9	73±1 (-58)	20.9±1.8 (-27)	40.8±7.2 (+67)
Flour	None	SDW	6.2	136±24 (-21)	21.0±1.3 (-27)	27.8±12.6 (+13)
		SAB	16.1	106±18 (-39)	29.2±6.2 (+2)	15.3±2.9 (-38)
		SPS	4.9	64±14 (-63)	26.0±3.2 (-9)	40.0±1.6 (+63)
	PD	SDW	6.5	149±22 (-14)	23.3±1.0 (-19)	33.3±7.0 (+36)
		SAB	13.1	114±4 (-34)	21.1±1.3 (-26)	30.2±7.0 (+23)
		SPS	9.6	127±28 (-27)	33.5±0.2 (+17)	28.1±2.2 (+15)
Rice bran						
	None	Control	0	617±26	94.6±3.0	57.5±1.1
	None	SDW	7.4	628±44 (+2)	163.9±13.4 (+73)	19.3±1.7 (-66)
		SAB	15.1	493±20 (-20)	99.3±11.4 (+5)	15.4±2.4 (-73)
		SPS	20.2	318±22 (-48)	90.0±8.4 (+5)	21.0±0.4 (-63)
	PD	SDW	28.7	671±50 (+9)	141.3±11.7 (+49)	22.3±3.0 (-61)
		SAB	29.8	524±18 (-15)	125.2±5.5 (+32)	16.1±1.5 (-72)
		SPS	19.4	334±8 (-46)	95.2±12.0 (+1)	21.9±3.1 (-62)
Brown rice						
	None	Control	0	284±18	50.7±9.2	34.0±0.4
Kernel	None	SDW	5.0	173±15 (-39)	34.7±9.4 (-32)	26.3±2.6 (-23)
		SAB	16.0	209±4 (-26)	28.0±10.6 (-45)	26.3±1.9 (-23)
		SPS	0.1	222±15 (-22)	26.6±4.1 (-47)	31.4±4.4 (-8)
	PD	SDW	12.6	183±7 (-36)	27.6±4.1 (-46)	29.0±2.3 (-15)
		SAB	18.9	121±10 (-57)	20.2±4.1 (-60)	23.1±2.5 (-32)
		SPS	8.6	127±28 (-55)	33.5±0.2 (-34)	28.1±2.2 (-17)
Flour	None	SDW	31.2	192±16 (-32)	35.0±5.1 (-31)	26.5±8.4 (-22)
		SAB	31.4	108±3 (-62)	31.4±4.5 (-38)	20.8±4.2 (-39)
		SPS	1.5	124±27 (-56)	30.9±3.3 (-39)	26.0±7.7 (-24)
	PD	SDW	6.2	175±18 (-38)	28.2±5.8 (-44)	29.2±0.0 (-14)
		SAB	13.4	153±15 (-46)	32.8±4.4 (-35)	17.1±2.7 (-50)
		SPS	10.3	121±20 (-57)	27.5±2.0 (-46)	21.2±0.1 (-38)

[1]: For an explanation of codes, see table 1.

[2]: Numbers between brackets refers to the changes compare to initial values

White rice kernels

For white rice kernels, phytic acid started at relatively low levels and disappeared from the solids with a concomitant release of soluble phosphorus in the medium (figure 1). Although the differences were small, phytase application led to significantly higher levels of phosphorus in the media. After grinding white rice to powder, soaking was even more effective in increasing soluble phosphorus. Interestingly, phytase did not lead to similar levels of phosphorus, while phytic acid was reduced to below detection level.



For explanation of codes, see Table 1

Figure 1: Changes of phosphorus (P) in white rice

Dry matter losses (table 2) of white rice kernels were smaller than from ground powder since they have less exposed surface area enabling diffusion. It appears that acid soaking leads to higher dry matter losses than the other soaking media. Calcium levels show some variations in losses but we cannot observe a significant trend, except that calcium appears to be lost in greater quantities than dry matter. Iron levels follow the same order of magnitude as calcium, with relatively higher losses from kernels than from powder. Zinc appears to follow another trend, with a number of apparent increases. In particular the phytase treatment results in higher zinc levels in the solid matter.

Rice bran

Levels of phytic acid in rice bran are considerably higher than in white rice. Acidic buffer reduced phytate and total phosphorus by about 50% with a concomitant release of soluble phosphorus into the medium (figure 2), while soaking in demineralized water had no effect. The application of phytase was far more effective, although at the very high initial phytate levels a higher phytase activity or exposure time might have been required to obtain a complete removal (Egli et al. 2003).

The dry matter losses (table 2) are the highest compared with the other rice fractions, reaching almost 30%. The mineral levels in bran, especially of calcium and iron, are about 4 times higher than in white and brown rice. Acidic buffer, and more so phytase soaking results in strong migration of calcium and iron into the medium. Zinc levels are about twice higher than in white and brown rice, and suffer equally as calcium and iron, from leaching into the soaking media.

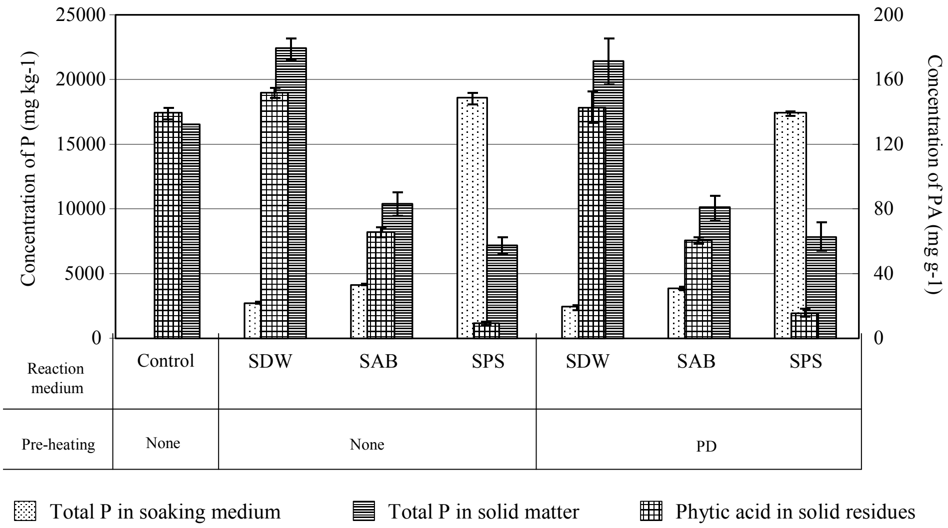


Figure 2. Changes of phosphorus (P) in rice bran

Brown rice

The results obtained with brown rice to a large extent paralleled those of rice bran. An interesting contrast was observed between the kernels and the powder obtained by grinding them (figure 3). In kernels, soaking in water and acid buffer gave similar results as in bran, i.e. water is not effective whereas acidic buffer extracts phytate phosphorus with release of soluble phosphorus into the medium. Phytase had no significant effect. In contrast, in ground powder, the phytase was highly effective resulting in removal of phytate below detection level.

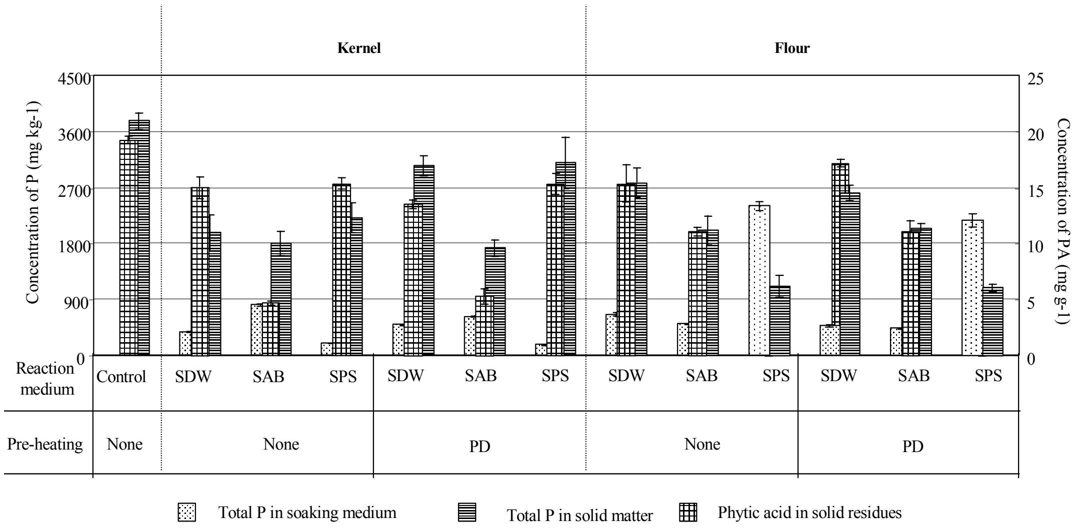


Figure 3. Changes of phosphorus (P) in brown rice

The dry matter losses (table 2) from whole kernels are of the same order as from white rice kernels. From powdered brown rice, much more dry matter is lost by soaking. Calcium levels in brown rice are higher than in white rice, but the relative losses are similar as a result of soaking. The same can be observed for iron and zinc.

DISCUSSION

For different fractions of rice, preheating and soaking conditions have diverse effects on contents of phytic acid and minerals. In order to distinguish between the effects of endogenous and microbial phytase during soaking, we attempted to inactivate endogenous phytase by preheating. The effect of dry preheating was negligible, probably because of very low levels of active endogenous phytase, and also because no significant structural (textural) changes took place in the kernel. This in contrast to the wet preheated rice; this was however, not useful as an experimental treatment because no separation between solids and liquids was possible anymore because of gelatinization; moreover, such a product would be less acceptable to the consumer.

Phytase treatments decreased phytic acid contents by degradation, which released phosphate from phytic acid to the reaction medium, so the concentration of phosphorus in the reaction medium increased (Wu et al. 2004). Soaking reduces phytic acid by two phenomena, i.e. enzymatic hydrolysis induced by endogenous phytase, and diffusion of water-soluble phytate (Perlas and Gibson 2002). The degradation by endogenous phytase was reported to be affected by pH and the temperature of medium (Sattar et al. 1989). Diffusion was reported to be affected by the nature of the phytate, which may be in the form of potassium, calcium or magnesium salts, and the pH of the medium (Mahgoub and Elhag 1998). It was reported that soaking in distilled water was more effective to remove phytic acid from pulses, than sodium bicarbonate (0.02%, w/v) (Vijayakumari et al. 1998). We propose that soaking in acidic buffer is more effective to decrease phytic acid than demineralised water from brown rice and rice bran, because the higher solubility of phytates in acidic solutions accelerates their migration into the soaking medium.

With regard to minerals in rice fractions, losses of minerals were mainly caused by diffusion into the soaking medium (Barampama and Simard 1995). Increases of mineral concentration are apparent, since they are related to a loss of total mass. Quantities of minerals lost are influenced by the soaking medium (e.g. pH, temperature) and also by complexion of minerals with other components, such as phytic acid, fibre and polyphenols. Only those minerals released from complexation will be soluble in the soaking medium (Lestienne et al. 2005b). Soaking caused a significant decrease of calcium in all rice fractions, except for rice bran soaked in demineralized water (12-63%). Our results were similar to Perlas and Gibson's, who also found that soaking rice flour (1-12 h, 30 ºC) in water would lead to 12-23% of loss of calcium (Perlas and Gibson 2002). For rice bran, treatment with commercial phytase and soaking in acidic buffer resulted in absolute losses of calcium ranging from 46 to 48% and from 15 to 20%, respectively, while the concentration of calcium on dry matter basis increased slightly (2-9%), which might be caused by considerable loss of mass (7-30%). These results could be explained by the low pH, and high temperature and/or degradation of phytic acid, which enable the leaching of calcium, while it was reported that the solubility of calcium at higher pH medium (>6) was low (Nestares et al. 2003). Soaking of white rice flour in water at 30ºC for 12 h would increase the concentration of iron (8%) (Perlas and Gibson 2002), while soaking at low temperature (24 h, 10ºC) led to decrease (19%). We suggest that increases at 30 ºC were apparent, and resulted from dry matter losses caused by fermentation or diffusion.

From the initial materials, acid soak of white rice would be adequate to dephytinize intact kernels. For bran, a very interesting opportunity will be to dephytinize with phytase, and dissolve a majority of minerals in liquid medium, to obtain a natural mineral enrichment that could be used in formulated foods. The data on brown rice vs. milled brown rice give a very clear illustration of the function of the testa and aleurone layers as a barrier to influx of e.g. phytase, and diffusive loss of matter. It has been reported that the presence of bran, which retards water penetration and therefore the leaching of solids (Bello et al. 2004), limits the passage of phytate and phytase (Lestienne et al. 2005c). The effect of the bran layer is also the main reason for the lower mass loss of brown rice kernel soaked in demineralised water than that in acidic buffer, which might be caused by breakdown of the outermost layer of bran in acidic solution which increased loss of solid mass. A similar situation were observed in dehulled beans (Aminigo and Metzger 2005; Bayram et al. 2004).

CONCLUSIONS

White rice, rice bran and brown rice behaved differently regarding losses of dry mass, minerals and phytic acid, when soaked with demineralized water, acidic buffer and phytase solutions after preheating. White rice contained 4 mg g^{-1} phytic acid which was removed by all treatments to below detection level. Dry matter losses due to soaking ranged from 2-20% with highest losses observed after soaking in acidic buffer. Whereas relatively high losses of calcium (12-63%) and iron (9-43%) were recorded, zinc losses were lower (0-38%). Bran contained 140 mg g^{-1} phytic acid which was removed best (-92%) by phytase treatment, followed by soakingin acidic buffer (-50%). Dry matter losses due to soaking were highest of all rice fractions (7-30%). Bran contains the highest levels of calcium (671 mg g^{-1}), iron (95 mg g^{-1}) and zinc (57 mg g^{-1}), and phytase treatment resulted in dissolution of 50-70% in the soaking medium. Brown rice contained 20 mg g^{-1} phytic acid which was removed best from kernels (75% of initial) by soaking in acidic buffer, but in flour by phytase application to below detection levels. Dry matter losses due to soaking ranged from 0.1-31% with highest losses observed after soaking in acidic buffer. Brown rice contains about two-fold higher levels of calcium, iron and zinc than white rice. However, losses due to soaking were similar as in white rice, for calcium (22-62%), iron (31-60%), and zinc (8-50%).

Phytase application is an effective method to rapidly remove of phytic acid while preserving dry matter and minerals in rice fractions.

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Chapter 5

Effect of soaking, germination and fermentation on phytic acid, total and in vitro soluble zinc in brown rice

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(submitted)

Abstract

Rice is an important staple food in Asian countries. In rural areas it is also a major source of micronutrients. Unfortunately the bioavailability of minerals, e.g. zinc from rice is low because it is present as an insoluble complex with food components such as phytic acid. We investigated the effects of soaking, germination and fermentation with the aim to reduce the content of phytic acid, while maintaining sufficient levels of zinc, in the expectation to increase its bioavailability. Fermentation treatments were most effective in decreasing phytic acid (56-96% removal), followed by soaking at 10°C after preheating (42-59%). Steeping of intact kernels for 24 h at 25°C had the least effect on phytic acid removal (<20%). With increased germination periods at 30°C, phytic acid removal progressed from 4 to 60%. Most wet processing procedures, except soaking after wet preheating, caused a loss of dry mass and zinc (1-20%). In vitro solubility as a percentage of total zinc in soaked rice was significantly higher than in untreated brown rice, while in steeped brown rice, it was lower ($P<0.05$). Fermentation and germination did not have significant effects on the solubility of zinc. The expected improvement due to lower phytic acid levels was not confirmed by increasing levels of in vitro soluble zinc. This may result from zinc complexation to other food components.

INTRODUCTION

In developing countries, cereals and legumes are the main dietary sources of macronutrients (carbohydrate, protein) as well as micronutrients such as iron, calcium and zinc (Lestienne et al. 2005b). In China, plant foods provide at least 50% of the dietary energy and nutrients (Ma et al. 2005), and rice is the most important staple food. Unfortunately, rice is not a good source of metabolizable micronutrients due to the presence of phytic acid. In plants, phytic acid is one of the main inhibitors of the availability of divalent cations like Fe^{2+}, Ca^{2+}, Mg^{2+} and Zn^{2+}. The phosphate groups of phytic acid (inositol hexakisphosphate) form stable complexes with such cations, thus preventing their bioavailability.

The bioavailability of minerals from foods is defined as the proportion of the minerals that can be absorbed and utilised within the body (Larsson et al. 1997; Lestienne et al. 2005a). Solubility of minerals, pH of intestinal lumen, dietary factors and residence time at the absorption site influence the bioavailability of minerals (Larsson et al. 1997). The latter could be predicted by molar ratios of phytic acid to minerals, HCl (hydrochloric acid)-extractability and in vitro solubility of minerals. Ma (2007) recently proposed that when phytate and zinc are present in a molar ratio of 16:1, the zinc is no longer bioavailable. Typical ratios found for rice are: 4-15 and 61-74 for white and brown rice, respectively.

Since the health benefits of improving the bioavailability of minerals in rice are considerable, we have undertaken a systematic study consisting of three parts: 1) a screening of a wide range of rice varieties and growing conditions with respect to phytate and mineral contents; 2) a dry fractionation study aimed at identifying optimum conditions for removing phytate while retaining nutrients; and 3) a wet processing study with the same aim. The first study was recently published (Liang et al. 2007) and reported a wide variation in both minerals and phytate. The optimum combination (highest mineral concentration and lowest phytate content) did not provide a significant increase in predicted mineral bioavailability. In the second study (Liang et al. accepted), it was found that milling leads to considerable losses of both phytic acid (minerals inhibitor) and minerals. Since for example, iron and phytate are similarly distributed in the kernel, a dry fractionation would provide only limited opportunities for their separation. On the other hand, zinc is more evenly distributed in the kernel and could be separated from phytic acid. Wet processing provides a third technical option to improve the bioavailability of minerals. Such food processing methods, which can be used both at the industrial scale and in the household, appear to be promising to combat deficiencies of iron and zinc (Gibson et al. 2000). It was reported that wet processing (including soaking, germination and fermentation) leads to a reduction in phytic acid, and could thus improve bioavailability of minerals in cereals and legumes.

Soaking of millet, soya bean, maize, sorghum, and mung bean at 30°C for 24 h decreased the contents of phytic acid by 4-51% (Lestienne et al. 2005b; Lestienne et al. 2005c), and soaking of sorghum flour (80% extraction) at room temperature for 24 h reduced phytic acid levels by 16-21% (Mahgoub and Elhag 1998). Soaking of pounded maize for 1 h at room temperature already led to a reduction of phytic acid by 51% (Hotz et al. 2001).

The use of moist conditions to stimulate germination is another technique to try and reduce phytate levels. Germination of sorghum for 4 d reduced phytic acid by 68-87% (Mahgoub and Elhag 1998). Badau reported that with longer germination times, HCl-extractability of calcium, iron and zinc in pearl millet was increased by 2-16%, 15-45% and 12-25%, respectively (Badau et al. 2005).

Sourdough fermentation (30 °C for 4 h), led to a reduction of 60% of phytic acid in whole wheat flour with a >30% increase of iron and zinc in vivo absorption in rats (Leenhardt et al. 2005; Lopez et al. 2003). After 12 h accelerated fermentation, 60% of phytic acid in sorghum was degraded (Mahgoub and Elhag 1998).

In summary, although a complete removal of phytic acid was not reported, wet processing technologies can help reduce phytic acid. To our knowledge, there is little information in literature on wet processing of rice.

Brown rice contains more minerals than white rice. Compared to white rice, consumption of brown rice would increase the intake of iron and zinc by a factor of 3 and 1.7 respectively (Doesthale et al. 1979; Heinemann et al. 2005; Kennedy et al. 2002). We therefore selected brown rice for this study. The objectives of the present study were 1) to analyse the effects of wet processing (soaking, germination and accelerated lactic fermentation) on the mass balance and levels of minerals and phytic acid in brown rice, and 2) to study the effect of phytic acid contents on the solubility of zinc in wet processed materials.

MATERIALS AND METHODS

Materials

Brown rice (Kenjian 90-31, cultivated in Heilongjing province and harvested in 2003) was collected from Beijing Huateng Model Rice Mill Company (Beijing, China).

Soaking

Brown rice was soaked at 10 °C at a ratio of rice to soaking solution of 1:5. Prior to soaking, the rice was either heated as is, in a drying oven for 30 minutes at 100 °C (dry preheated), or mixed with demineralized water (1:1, w/v) and autoclaved for 10 min at 115 °C (wet preheated). After this step, the rice was soaked with demineralised water (1:5, w/v). The mixture was either left as is (pH 5.9) or adjusted with 5 N HCl to pH 3.5. Rice was soaked for either 1 or 7 days. After soaking, the total mixture was centrifuged at 5000 *g* (10 min). The pellet was dried and stored for further analysis. Table 1 summarizes the different soaking conditions used.

Table 1: Preheating and soaking conditions

Treatment Code	Preheating		Soaking medium		Soaking time (d)	
	Dry heated [a]	**Wet heated [b]**	**Natural demineralized water (pH 5.9)**	**Acidic solution [c]**	**1**	**7**
SDN	×		×			×
SDA	×			×		×
SWN		×	×		×	
SWA		×		×	×	

[a]: Brown rice was heated in drying oven at 100°C for 30 min before soaking
[b]: Mixture of brown rice and demineralized water (1:1, w/v) was heated in autoclave at 115°C for 10 min.
[c]: pH of mixture was adjusted to 3.5 with 5 N HCl before soaking

Steeping and germination

Steeping and germination were carried out following the procedure developed by Capanzana and Buckle (1997). Approximately 30 g of brown rice was soaked in 150 mL demineralised water in plastic boxes at 25 °C. Steeped brown rice was sampled at the intervals of 4, 8, 12 and 24 h. After steeping, brown rice was separated by decanting and placed in plastic boxes, covered with punctured lids and left to germinate for 72 h at 30 °C. Samples were taken at 12, 24, 36, 48 and 72 h.

Fermentation

Mixtures of brown rice and demineralized water (ratio 1:5, w/v) were fermented naturally for 24 h at 30°C. Starting on the second day and after each consecutive day, a fresh mixture was inoculated with 10% of the water of the previously fermented mixture, in order to obtain an accelerated fermentation by enrichment of acidifying microbiota (Nche et al. 1994).

All treatments were carried out in duplicate. After each treatment, the rice was transferred to glass dishes together with liquids and dried at 70°C until constant weight. Dried materials were stored in sealed plastic bags at 4°C before chemical analysis.

Zinc analysis

Total zinc content: Rice samples were digested with hydrofluoric acid (40%, w/w) and concentrated nitric acid (65%, w/w) to allow the analysis of total zinc. Zinc contents were analysed using an inductively coupled plasma optical emission spectrometer (ICP-OES) (Elan 6000, Perkin-Elmer, Norwalk, CT). Each sample was digested and extracted in duplicates.

In vitro soluble zinc content: In vitro soluble zinc was defined as the relative amount of zinc that becomes soluble after enzymatic treatment. Rice samples were digested following the enzymatic degradation procedure described by Kiers (2000). Then, mixtures were centrifuged at 5000 *g* for 15 min at 4°C. The resulting supernatant was filtered (0.45 µm membrane, FP 030/3) and frozen until further analysis. Zinc levels were analysed as described earlier.

Phytic acid

A 100 mg amount of sample (previously dried and ground) was placed in a glass tube and mixed with 10mL of 18.25 g dm^{-3} HCl containing 50mg dm^{-3} cis-aconitate (internal standard). The mixture was boiled at 100°C for 15 min in a water-bath. Next, samples were transferred into 2 mL Eppendorf tubes and centrifuged at 21 000 *g* for 10min. A 0.1 mL volume of the supernatant was homogeneously mixed with 0.1mL of 18.25 g dm^{-3} HCl and 0.8mL of MilliQ water (Millipore, Billerica, MA, USA) and from this mixture 200 µL were immediately transferred to HPLC phials.

Phytic acid was determined by high-performance liquid chromatography (HPLC) (Bentsink et al. 2003) using a Dionex (Sunnyvale, CA, USA) DX300, ICS2500 system with a suppressed conductivity detector range of 10 µS. An AS11 chromatographic column (Dionex) with a guard column was used. All analyses (20 µL injection volume) were carried out at room temperature. The eluents (flow rate 1 mL min^{-1}) used were as follows: 0-5min, 0.2 g dm^{-3} NaOH; 5-15min, linear gradient of 0.2-4 g dm^{-3} NaOH; 15-20 min, 20 g dm^{-3} NaOH; and finally 20-35min, 0.2 g dm^{-3} NaOH.

Statistical analysis

Data were analysedyzed with SPSS 10.0 for windows. Significance was tested at a 5% level using an independent-samples *t*-test.

RESULTS

Effect of wet processing on dry matter, phytic acid and zinc

The various wet processing treatments were tested for their efficacy on decrease of phytic acid, while retaining micro- and macronutrients. In figure 1, an overview is presented of the different results obtained.

Dry Matter: Wet treatments led to a loss of dry matter between 7-16%. Mass loss in accelerated fermented brown rice was 9-12%. Soaking led to a higher mass loss than other treatments, especially soaking after wet preheating, with losses up to 16%. Mass loss during steeping and sprouting ranged from 7-13%.

Decrease of phytic acid: Compared to untreated brown rice, wet processes (soaking, steeping and fermentation) significantly decreased phytic acid levels. However, they varied in their ability to reduce phytic acid levels, which can be derived from the retention of phytic acid in samples (5-96%). Fermentation was clearly the most effective: decreasing phytic acid with 53% up to 95%. Steeping of intact grains as a first step of germination was not significantly effective: only a reduction of 14-28% was obtained. During fermentation as well as germination, decrease of phytic acid progressed with time. No significant difference was observed between dry and wet preheating ($p<0.05$).

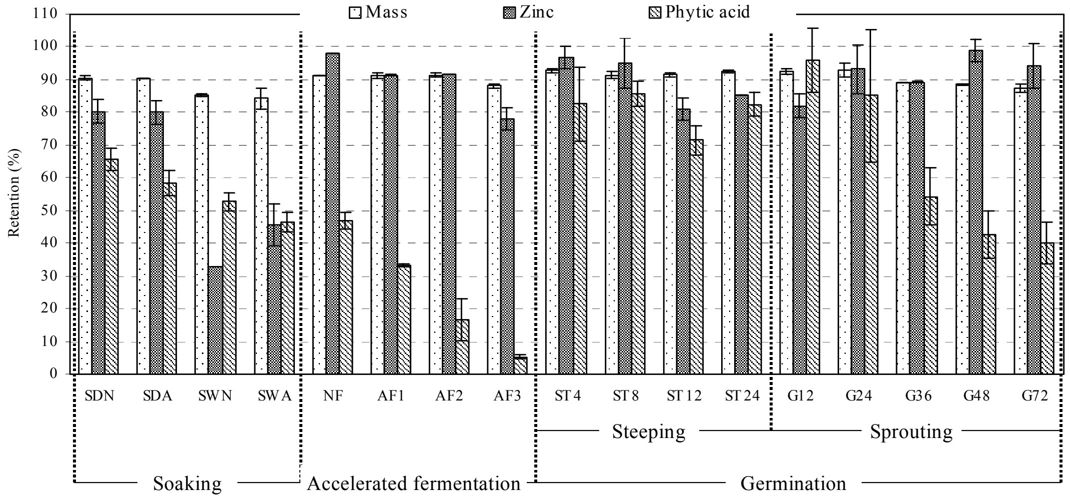

Figure1. Retention of mass, zinc and phytic acid in brown rice upon wet processing

Abbreviations: SDN, SDA, SWN, SWA: see Tab.1; NF: natural fermentation without starters addition at 30 °C for 24 h; AF1/AF2/AF3: accelerated fermentation with one/two/three cycles of starter enrichment for 24 h and fermented at 30 °C for 24 h; ST4/ST8/ST12/ST24: brown rice was steeping for 4, 8, 12 and 24 h respectively; G12/G24/G36/G48/G72: brown rice was germinated for 12, 24, 36, 48 and 72 h respectively; Retention: Percentage of dry mass/zinc/phytic acid in certain quantities of brown rice after to before treatments

Retention of zinc: Results also varied with the different wet processes applied. In general, zinc retention was between 80 and 99%. Only wet preheating led to a considerable loss of zinc (ca 60%). In most wet treatments of brown rice, the retention of zinc was at similar levels as the retention of dry matter, which was in the range of 80-93%. Rice soaked after wet preheating formed the exception, with dry matter retention at 33% and 46%, after soaking in demineralized water and acidic solutions, respectively. Retention of zinc in brown rice soaked after dry heating was about 80%, which was much higher than after wet preheating.

Contents of phytic acid and zinc in treated brown rice

The relative losses of phytic acid, zinc and dry matter resulted in changes of absolute concentrations that are of nutritional relevance. Table 2 presents the concentration of phytic acid and zinc in treated materials.

Table 2: Effect of soaking, germination and fermentation on brown rice [a]

Treatments	Methods [b]	Phytic acid [c] (mg g^{-1})	Zinc [d] (mg kg^{-1})
Untreated	None	12.5 ± 0.2	19.6 ± 1.4
Soaking	SDN	9.1 ± 0.5	16.5 (16, 17)
	SDA	8.0 ± 0.5	16.4 (16, 17)
	SWN	7.7 ± 0.4	7.1 (7, 7)
	SWA	6.9 ± 0.4	10.0 (9, 11)
Natural and Accelerated Fermentation	NF	6.4 ± 0.3	19.9 (20, 20)
	AF1	4.5 ± 0.1	18.5 (19, 19)
	AF2	2.3 ± 0.9	18.6 (19, 19)
	AF3	0.7 ± 0.1	16.4 (16, 17)
Germination: (1) Steeping	ST4	11.1 ± 1.5	19.3 (19, 20)
	ST8	11.7 ± 0.5	19.3 (16, 23)
	ST12	9.7 ± 0.6	16.4 (16, 17)
	ST24	8.4 ± 0.5	17.1 (17, 17)
Germination: (2) Sprouting	G12	12.9 ± 1.3	16.4 (16, 17)
	G24	11.4 ± 2.7	18.5 (17, 20)
	G36	7.6 ± 1.2	18.6 (19, 19)
	G48	6.0 ± 1.0	20.7 (20, 21)
	G72	5.7 ± 0.9	20.0 (19, 21)

[a]: all data expressed on dry matter basis

[b]: abbreviations of treatments were the same as in figure1

[c]: contents of zinc in untreated brown rice and contents of phytic acid in all materials: mean ± standard deviation (n=4)

[d]: contents of zinc: average of results of replicates of treatments (values of replicates).

Phytic acid: The level of phytic acid in untreated brown rice was 12.5 mg·g^{-1}, while it ranged from 1 to 13 mg g^{-1} in treated brown rice. After soaking, the phytic acid was around 7-9 mg g^{-1} with wet preheated rice having lower levels than dry preheated. Rice fermented with enrichment starter (AF3) had the lowest phytic acid content (0.7 mg g^{-1}), but natural fermentation (NF) also reduced the initial phytic acid level by 50%. During germination, contents of phytic acid slightly decreased from 11.7 to 8.4 mg g^{-1} during steeping phase, and decreased further from 12.9 to 5.7 mg g^{-1} during sprouting over a period from 12 h to 72 h.

Zinc: The zinc level in initial brown rice was 19.6 mg kg^{-1}. It ranged between 16-21 mg kg^{-1} in treated rice, except in brown rice soaked after wet heating, which contained less than 10 mg kg^{-1}.

Molar ratios of phytic acid to zinc ([PA]/[Zn]) and in vitro solubility (IVS) of zinc

The molar ratio of phytate to zinc is assumed to provide an indication of the bioavailability of zinc. In untreated brown rice this ratio was 63, while after processing molar ratios of [PA]/[Zn] varied from 4 to 107. The lowest ratio was obtained by fermentation (AF3), and the highest in soaked rice after wet preheating. The amount of IVS-zinc of treated brown rice is assumed to provide an indication of the amount of zinc available for absorption in vivo. The IVS is expected to be more relevant than the phytate to zinc ratio, which is only based on contents. Untreated brown rice had 7.3 mg kg^{-1} IVS-zinc, and after the various treatments IVS zinc levels ranged between 4.2-8.3 mg kg^{-1} (table 3).

Table 3: In-vitro solubility of zinc and molar ratios of phytic acid to zinc in treated materials

Treatments	Methods [a]	In vitro soluble zinc (mg kg^{-1}) [c]	Solubility (%)	[PA]/[Zn] [d]
Untreated	UR	7.3 ± 0.2	37.5	62.8
Soaking	SDN	7.7 (8, 8)	46.9	54.3
	SDA	6.7 (4, 9)	40.9	48.0
	SWN	5.8 (5, 7)	33.8	106.8
	SWA	5.7 (5, 6)	56.8	68.0
Natural and Accelerated Fermentation	NF	6.0 (3, 9)	30.2	31.7
	AF1	6.8 (7, 7)	36.8	24.0
	AF2	7.4 (5, 10)	40.0	12.2
	AF3	4.1 (3, 5)	24.8	4.2
Germination: (1) Steeping	ST4	5.4 (5, 6)	28.2	56.6
	ST8	5.5 (5, 6)	28.6	59.7
	ST12	4.6 (3, 6)	28.0	58.3
	ST24	6.6 (5, 8)	38.4	48.4
Germination: (2) Sprouting	G12	6.6 (6, 7)	40.0	77.5
	G24	4.2 (3, 5)	22.7	60.7
	G36	6.2 (6, 7)	33.1	40.2
	G48	6.1 (5, 8)	29.5	28.5
	G72	8.3 (8, 8)	41.3	28.1

[a]: abbreviations of treatments were the same as in figure1

[b]: contents of zinc: average of results of replicates of treatments (values of replicates)

[c]: calculated on total contents of phytic acid and zinc in treated materials

[d]: expressed on dry matter basis

DISCUSSION

Given the important role of rice in the diet both as a source of macro- and micronutrients, an improvement of the bioavailability of zinc and iron is needed. Our studies indicated that selection of rice varieties (Liang et al. 2007), or optimization of dry processing (milling) had limited promise for improvement of mineral availability. We therefore investigated the potential effect of wet processing. We aimed at identifying wet operations that can be used in combination with optimum growing and dry fractionation procedures. For the same reason, our analysis of the samples was limited to three main factors: phytate, zinc and dry matter. Other micronutrients such as iron are equally important but in this study we focused on zinc.

Our results with wet processes showing that they could significantly decrease phytic acid in brown rice agree well with reports about similar treatments on other cereals, such as fermentation of white rice flour (Reddy and Salunkhe 1980), and steeping and germination of oats or corn (Fageer et al. 2004; Larsson and Sandberg 1995). We found that soaking, germination and fermentation have a different efficacy in reducing the content of phytic acid. Whereas natural fermentation (NF) only removes half of the phytic acid, accelerated fermentation (AF) was more effective. In sorghum, soaking and natural fermentation had a similar impact on phytic acid (Mahgoub and Elhag 1998) which is in line with our results with NF. Germination is more effective than soaking. Decreases caused by fermentation and germination are mainly based on the action of enzymes, while in soaking a combination of diffusion and enzymatic action is expected (Henderson and Ankrah 1985; Mahgoub and Elhag 1998). Other studies also proposed that the presence and activity of endogenous phytase was the main factor leading to a reduction of phytic acid during soaking (Lestienne et al. 2005b). In a study by Carlson and Poulsen (2003) in which heated and non-heated barley and wheat were soaked, reduction of phytic acid was clearly higher in the non-heated treatment. Unfortunately, the heat treatment was not specified, so we cannot compare their results with ours. They ascribed the difference in phytate degradation to the inactivation of endogenous phytase. This again points at the role of endogenous phytase. We did not obtain such differences between our untreated and preheated samples, which may be due to the use of different grains and a different extent of heat treatment; in any case, we found phytase activity in both untreated and preheated rice (data not shown). Our results demonstrate that the reduction of phytic acid increased with germination time, which agrees with previous studies reporting that the activity and/or production of phytase increased during steeping (Henderson and Ankrah 1985; Larsson and Sandberg 1995). We observed similar trends with fermentation. Abdalla and Towo suggested that enzymatic hydrolysis of phytic acid by both endogenous phytase and microbial phytase (lactic acid bacteria can produce significant levels of phytase), may account for most of the loss of phytic acid during fermentation (Abdalla et al. 1998; Leenhardt et al. 2005; Towo et al. 2006). Our observation of low phytic acid contents after fermentation is similar to results obtained with fermented whole wheat flour (Lestienne et al. 2005b). The results indicated that both endogenous phytase and microbial phytases contributed to a reduction of phytic acid in fermented brown rice. From table 4 we observed that NF was much less effective in acidification and phytate removal than fermentation with cycle 3 starter (AF3). This could be due to a more rapid pH decrease and/or higher phytase activity. In addition to the influence of reaction conditions and ingredients used, factors such as enzymatic degradation, effect of pH on solubility and diffusion, could all be involved in the overall effect of the fermentation.

Table 4: Initial and final pH values after natural, and accelerated fermentation of brown rice

Treatment [a]	Initial pH [b]	Final pH [b]
NF	7.6 (7.6, 7.6)	5.5 (5.5, 5.6)
AF1	6.6 (6.5, 6.6)	5.3 (5.2, 5.4)
AF2	6.3 (6.2, 6.3)	5.2 (5.0, 5.4)
AF3	5.7 (5.6, 5.9)	4.8 (4.5, 5.1)

[a]: abbreviation was the same as in figure 1.

[b]: average (values of replicates)

The retention and solubility of individual minerals is also affected by process conditions. For zinc, only soaking after wet processing resulted in significantly lower retention compared with the other treatments. This can be explained by the uniform distribution of zinc in the grain and the effect of wet preheating. Whereas dry preheating only results in minor physical damage such as fissures (or cracks) in the kernels, wet preheating induced swelling of the kernel and gelatinization. The swollen kernel would allow better diffusion and we expect this to be the main reason for the higher loss of zinc and dry matter in soaked brown rice after wet preheating. In fermented brown rice, zinc losses ranged from 1-20%.

We expected that with lower molar ratios of [PA]/[Zn], the in vitro solubility of zinc would increase, but this relation was not significant. This discrepancy was also observed by authors who studied other cereals such as sorghum, millet and millet bran fractions (Lestienne et al. 2005a; Matuschek et al. 2001). Possibly, the molar ratio of [PA]/[Zn] must be very low (possibly <0.2) to achieve increased zinc solubility. Theoretically, one mole of phytic acid could bind four moles of zinc. Under the conditions used, soaking, germination or fermentation are not able to completely eliminate phytic acid, so the residual phytate might still interfere with zinc solubility. Another explanation is that a prediction of solubility of minerals is not feasible based on phytic acid to mineral ratios only. For instance, it was reported that lower inositol phosphates (inositol mono-, bi-, tri- and tetraphosphates) produced during fermentation, increase the capacity of binding minerals of higher-phosphorylated inositol phosphates (IP6 and IP5: *myo*-inositol pentaphosphate), although they were present at a low level (Sandberg et al. 1999). In addition, all forms of zinc phosphate are poorly soluble. Similar to polyphenols in sorghum and millet (Matuschek et al. 2001; Towo et al. 2006), other matrix components that limit in vitro solubility, such as dietary fibre, can be present in brown rice. Little is known about the affinity of such components to minerals. Their inhibition might be even more effective than phytic acid. Clearly, these hypotheses need further study.

Another aspect that needs further attention is the validity of in vitro solubility of zinc as an indicator for in vivo bioavailability. Although it has been demonstrated that the addition of phytic acid to e.g. white bread (Sandberg et al. 1993), results in a strong decrease of in vivo human zinc uptake, the situation for rice may be different, and more comprehensive data for solubility and in vivo uptake from food matrices are urgently needed.

From this study, we conclude that neither soaking, germination, nor fermentation significantly improve the apparent bioavailability of minerals in rice. It may be of interest to combine wet processing methods including phytase treatment with other approaches such as the application of uptake enhancers to improve the availability of minerals in rice. More research will be required to understand the effects of matrix components, and to explore opportunities for combined process approaches.

CONCLUSION

Soaking, germination and fermentation, led to a varying decrease of phytic acid in brown rice. The most effective approach (AF3) could reduce 96% of total phytic acid and decrease the molar ratios of phytic acid to zinc lower than 5. Wet processing (except soaking after wet heating) led to 1-20% of mass loss, and a similar loss of zinc as well.

The sharp decrease in ratio would suggest that especially fermentation would be a successful method to increase zinc bioavailability. However, results from in vitro solubility measurement of zinc showed little improvement over untreated brown rice. This could result from the presence of matrix components such as fibre or phosphates, leading to the formation of insoluble zinc complexes. It remains to be investigated to what extent this would affect IVS-solubility and bioavailability.

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Chapter 6

In vitro solubility of calcium, iron and zinc in relation to phytic acid levels in common rice-based consumer products in China

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(submitted)

Abstract

Rice-based products are widely consumed in China. In vitro solubility of calcium, iron and zinc in relation to phytic acid (PA) levels were studied in 30 commercially available rice-based products, including primary processed (brown, white, and germinated rice) and intensively processed (noodles, crackers, infant food) ones. Primary processed products had high molar ratios of phytic acid to minerals. Whereas [PA]/[Ca] values were less than 5, [PA]/[Fe] and [PA]/[Zn] were quite similar and ranged from 5-74 with most values between 20-30. Brown rice had much higher calcium (Ca), iron (Fe) and phytic acid levels compared with white rice. In intensively processed rice products, the [PA]/[mineral] molar ratios were generally lower. Rice noodles had the lowest phytic acid and the broadest range of mineral contents and in particular the highest levels and solubility of calcium. In rice crackers, the levels and solubility of minerals were similar as in rice noodles. In infant foods, the highest levels of minerals were observed. This is due to mineral fortification. Because of phytic acid levels in infant foods, high mineral levels did not always result in their higher solubility. Our data show that at present, neither primary nor intensively processed rice-based products are good dietary sources of minerals. Improvements should be attempted by dephytinization, mineral fortification or – preferably - combinations thereof.

INTRODUCTION

Together with maize and wheat, rice is a major food cereal in China with an estimated production of 187 million tons in 2004. About 95% of harvested rice is used for consumer food products either as white rice or as processed foods (e.g. noodles) (FAO 2006). Rice serves as the major dietary source of energy, protein, thiamine, riboflavin, niacin, iron and calcium (Juliano 1997; Kennedy et al. 2002). It was reported that in China, during the period 1997-2001, rice products supplied about 30-40% of the dietary energy intake (FAO 2004; Kennedy et al. 2002). In 2002 and 2003, intake of rice and rice products in China was 238 and 215 g/capita/day, respectively, which supplied 35% of total energy intake (FAO 2006; Wang 2005). This number is even higher in rural areas: estimates from 2002, give an intake of rice and rice products of 246 g/capita/day.

In China, iron deficiency related anaemia is quite common: it affects ca 30% of pregnant and lactating women. This situation did not improve by changes of the dietary structure (increased consumption of legumes and vegetables) aimed at increasing iron intake (Wang 2005). The main reason for iron deficiency anaemia and mineral malnutrition in China and other predominantly rice consuming countries is related to the poor bioavailability of iron and other important micronutrients (calcium, zinc). Ma recently reported that, while Chinese rely for ca 60% of their mineral intake on rice and other plant derived foods, the presence of phytate, a very potent inhibitor of mineral bioavailability, causes a low bioavailability of the minerals concerned (Ma 2007). In order to predict the bioavailability of minerals, molar ratios of phytic acid to minerals have been used as indicator (Adeyeye et al. 2000; Fordyce et al. 1987; Grewal et al. 1999; Hira and Kaur 1993; Ma et al. 2005). Bioavailability levels could also be related to the amount of minerals recovered after in vitro digestion of products with gut enzymes (Glahn et al. 2002; Kiers et al. 2000; Larsson et al. 1997).

Ma proposed that at a molar ratio of [PA]/[Fe]>1, iron uptake was inhibited. In earlier studies we reported typical values of [PA]/[Fe] of ca 50 in brown rice (predicted bioavailability <5%).Where increasingly, information becomes available on the bioavailability of trace elements in unprocessed products, information on bioavailability of minerals in processed products is still scant. In a previous paper, we demonstrated that especially wet processing like fermentation could lead to a substantial reduction in phytate levels (Liang et al. 2007).

According to the extent of processing, we distinguished primary and intensively processed rice-based products. Primary processed products include brown, white (or polished) and germinated rice and still have the kernel shape. Of these, white rice is the most important consumer product. Although brown rice and germinated rice contain higher levels of nutrients, they are not popular with the public because of their darker colour and unaccepted sensory properties (Huang 2004). Intensively processed products include rice noodles, rice crackers and rice-based infant foods, and mainly originate from further processing of white rice. Whereas several studies addressed process innovations for improved sensory quality (Lu et al. 2005; Park et al. 2001; Wang et al. 2003; Zhu 1990), only Ma included several rice products in a survey of minerals and phytic acid in common Chinese foods (Ma et al. 2005).

In previous studies we reported the natural variation in phytate and mineral levels in Chinese rice (Liang et al. 2007), and the efficacy of dry fractionation and wet processing in improving mineral bioavailability (Liang et al. submitted-a, b; Liang et al. accepted).

With the present study, we aim to gain understanding of the effect of standard commercial processing on the mineral contents and bioavailability of commercial rice products. To this end we collected 30 representative rice-based products from commercial outlets in China. All samples were analysed for their contents of minerals and phytic acid. We also assessed the in vitro solubility of minerals after enzymatic digestion.

The objectives were to 1) analyse levels and in vitro solubility calcium, iron and zinc in different rice-based products and their relation to levels of phytic acid; and 2) evaluate the suitability of rice products as dietary sources of minerals or as carriers for mineral fortification.

MATERIALS AND METHODS

Sample collection

30 commercial rice-based solid products with a shelf-life longer than 6 months were purchased at three supermarkets in Beijing. A description of these products is presented in table 1.

Table 1: Commercial consumer rice products investigated

Product Category (number of samples)	Processing methods	Moisture contents ($100g^{-1}$) Average	Range	Main ingredients
Brown Rice [a] (3)	Dehulled only	11.5	10.2 - 12.7	Brown rice
Germinated rice (1)	Brown rice was germinated till the sprout length was 0.5 - 1.0 mm (Huang, 2004)	26.3	----	Germinated rice
White rice [b] (8)	Milling and polishing to remove the outer layer of brown rice and obtain nice appearance and edible quality (Ruan, 2005)	12.9	11.6 - 13.5	White rice
Short-grain (5)	----	12.8	11.6 - 13.5	White rice
Long-grain (3)	----	12.9	12.6 - 13.2	White rice
Rice noodles (7)	----	11.4	10.2 - 13.0	----
Group 1 [c] (4)	White rice soaked in water, ground with or without water, steamed, extruded, cooled and dried (Zhu, 1990)	11.9	10.7 - 13.0	Rice and water
Group 2 [d] (3)	White rice together with other ingredients, such as starch or soy protein soaked or not soaked in water, ground, steamed, extruded, cooled and dried	10.6	10.2 - 11.0	Rice, water, starch, *Amorphophallus rivieri* flour and soy protein
Rice crackers (7)	Rice mixed with water, pulped, moulded, puffed, and baked (Wang et al., 2003)	2.3	1.2 - 3.5	Rice, sugar, oil, salt and soy sauce
Infant foods [e] (4)	White rice ground to powder, roasted, enzyme-treated, drum dried and formulated with other ingredients (Perez-Conesa et al., 2002; Zhao and Liu, 2004)	4.0	3.3 - 4.8	Ingredients varied according to products for infants' age, and for different manufacturers

a: all brown rice samples were short-grain
b: white rice samples were divided into two groups according to the length of kernel
c: ingredients of rice noodles are white rice and water, no other cereal materials added
d: other cereal materials were used together with rice
e: all infant foods were fortified with calcium, iron, zinc and some vitamins as stated on product labels

Analysis of total and in vitro soluble minerals

For analysis of total contents of calcium, iron and zinc, 1 g (accuracy 0.0001 g) sample was wet digested with nitric acid (HNO_3, 65 %) and perchloric acid ($HClO_4$, 60 %) following the procedure of the AOAC 975.03 (Horwitz 2000). In vitro soluble minerals were measured in the supernatant after enzymatic digestion of suspended food samples. Enzymatic digestion followed the procedure of Kiers et al. (2000). After digestion, the reaction mixture was centrifuged at 3600 *g* at 4 ºC for 15 min and the supernatants were filtered through a 0.45 µm membrane. Calcium, iron and zinc in acid digests and supernatants of enzymatic digests were determined with an inductively coupled plasma optical emission spectrometer (ICP-OES) (Optima 2000, Perkin-Elmer) (Bentsink et al. 2003). All samples were digested and analyzed in triplicates.

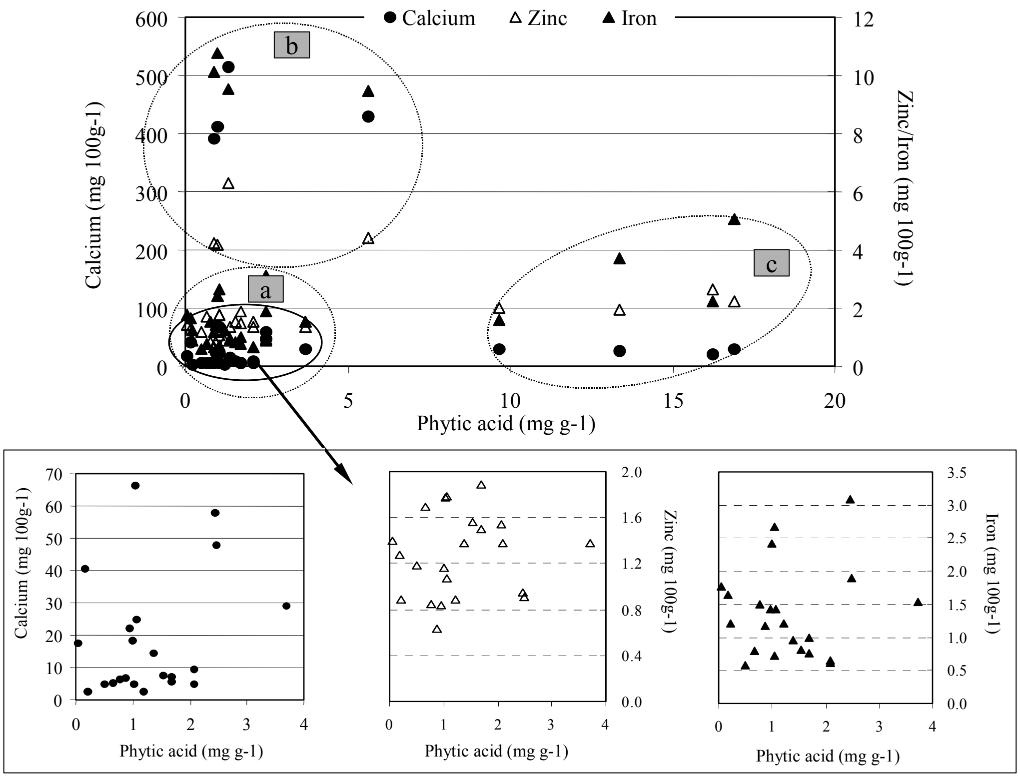
Contents of phytic acid

Phytic acid contents were analysed in triplicates, using the China National Standard Analysis Method following the procedures described by Ma et al. (Ma et al. 2005).

RESULTS

Phytic acid, calcium, iron and zinc, and molar ratios of phytic acid to minerals

Phytic acid, calcium, iron and zinc levels of all samples were mapped in figure 1. In this figure, samples could be clustered into three groups a, b and c as follows:



Each sample was in one of the three groups as a) white rice based products; b) brown rice based products; c) minerals fortified products

For better comparison, contents of phytic acid and minerals in most of the white rice based products were presented in enlarged figures below.

Figure 1: Levels of phytic acid and minerals (calcium, iron and zinc) in consumer rice products

Group a: low concentrations of both phytic acid and minerals: This group had phytic acid levels lower than 3.7 mg g^{-1} (wet weight) and calcium, iron and zinc levels below 66, 3.1 and 1.9 mg $100g^{-1}$, respectively. Twenty-two samples, including all white rice samples, rice noodles and rice crackers, can be found in this group. Concentrations of calcium were in the range of 2-66 mg $100g^{-1}$. They were lower than 20 mg $100g^{-1}$ in sixteen samples and ranged from 22-66 mg $100g^{-1}$ for the other six samples. Sixteen samples had concentrations of iron in the range of 0.6-1.5 mg $100g^{-1}$, and the other six contained 1.5-3.1 mg $100g^{-1}$. Eighteen samples had concentrations of zinc ranging from 0.8 to 1.6 mg $100g^{-1}$, three in the range 1.7-2.0 mg $100g^{-1}$, and one lower than 0.6 mg $100g^{-1}$.

Group b: low concentrations of phytic acid and high levels of minerals: This group consisted of four infant foods, fortified with calcium. Samples in this group had similar levels of minerals (about 450 mg $100g^{-1}$, 10 mg $100g^{-1}$ and 4 mg $100g^{-1}$ for calcium, iron and zinc, respectively), and different levels of phytic acid (0.9-5.9 mg g^{-1}) resulting from variously pre-treated ingredients. Consumption of 50 g food from this group would result in about 200-260 mg of calcium intake; 5 mg of iron and 2-3 mg of zinc; but at the same time, 50-300 mg of phytic acid would be consumed.

Group c: high concentration of phytic acid and low levels of minerals: This group represents brown and germinated rice. Levels of calcium, iron and zinc were 20-40 mg $100g^{-1}$, 2-6 mg $100g^{-1}$ and 2-3 mg $100g^{-1}$ respectively, and phytic acid levels were 10-17 mg g^{-1}. Although the consumption of rice products from this group will result in mineral intake, a considerable intake of phytic acid takes place at the same time.

Table 2 presents the levels of phytic acid, calcium, iron and zinc and the molar ratios of phytic acid to minerals in the various product categories. Compared with the other categories, infant foods had higher levels of minerals because of fortification. Among the other five categories, the highest levels of calcium and zinc occurred in germinated rice, and the highest levels of phytic acid and iron in brown rice. White rice had the lowest contents of calcium and iron. Noodles and crackers had similar mineral levels. Phytic acid levels in noodles and crackers were similar to those of white rice, about 10% of brown rice. This indicated that milling brown to white rice significantly reduced phytic acid levels, but that further processing did not significantly affect phytic acid.

As shown in table 2, the molar ratios of phytic acid to minerals were different for the respective types of minerals and rice products. In all product categories, [PA]/[Ca] ratios ranged between 0.0 and 4.6. This was much lower and the range was narrower than for [PA]/[Fe] (0.2-61) and [PA]/[Zn] (0.3-74). Ratios of [PA]/[Ca], [PA]/[Fe] and [PA]/[Zn] in brown rice and germinated rice were much higher than in other products. Infant foods had the lowest ratio of [PA]/[Ca], likely due to the calcium added. In the category of noodles the largest variation of phytic acid to minerals ratio was observed. These variations result from diverse concentrations of phytic acid and/or minerals. Our data in table 2 indicate that food processing significantly influences phytic acid or/and minerals in rice noodles. Compared to brown rice, it was found that milling, polishing, germination and more intensive processing involving soaking, fermentation, and enzymic treatments, decreased the molar ratios of phytic acid to minerals.

Table 2: Levels of phytic acid and minerals and molar ratios of phytic acid to minerals in rice products (dry mass basis)

Processing extent	Categories of products	Phytic acid (mg g^{-1})		Ca (mg $100g^{-1}$)		[PA]/[Ca]	Fe (mg $100g^{-1}$)		[PA]/[Fe]	Zn (mg $100g^{-1}$)		[PA]/[Zn]
		Average	Range	Average	Range	Range	Average	Range	Range	Average	Range	Range
Primary processed	Brown Rice	17.5	14.9-19.4	28.6	24-33	3.1-4.6	4.2	2.6-5.8	28-61	2.6	2.2-3.0	61-74
	Germinated rice [a]	13.1	13.1	38.7	39	2.1	2.2	2.2	52	2.8	2.8	47
	White rice	1.6	0.6-2.4	6.8	5-11	0.6-2.8	0.8	0.7-1.2	7-29	1.8	1.4-2.2	4-15
	Short grains	2.1	1.8-2.4	7.7	5-11	1.3-2.8	0.9	0.7-1.2	14-29	1.8	1.6-2.2	9-15
	Long grains	0.8	0.6-1.2	5.5	5-6	0.6-1.4	0.8	0.7-0.9	7-12	1.8	1.4-2.0	4-6
Intensively processed	Rice noodles	1.2	0.0-4.1	27.8	3-75	0.0-3.1	1.8	1.1-3.0	0.2-21	1.4	1.0-2.0	0.3-27
	Group 1	0.8	0.0-1.5	28.3	3-75	0.0-0.6	1.9	1.2-3.0	0.2-12	1.5	1.0-2.0	0.3-10
	Group 2	1.9	0.2-4.1	27.0	3-46	0.0-3.1	1.6	1.4-1.9	0.9-21	1.3	1.0-1.5	1.3-27
	Rice cracker	1.4	0.8-2.6	26.7	6-59	0.3-0.8	1.9	1.2-3.2	3.5-11.1	0.9	0.7-1.2	8-27
	Infant foods	2.3	0.9-5.9	455.0	408-532	0.0-0.1	10.4	9.9-11.2	0.7-5.1	5.0	4.3-6.5	2.0-12.7

a: mean of duplicate measurements in one sample

In vitro solubility of calcium, iron and zinc in rice products

The phytate to mineral ratio provides a crude indicator for mineral bioavailability. The amount of minerals solubilized after in vitro digestion of a sample are closer to the in vivo situation and may therefore be more predictive for true bioavailability.

Table 3: In vitro solubility (% of total content) of calcium, iron and zinc in rice products

Products	Calcium		Iron		Zinc	
(number of samples)	Average	Range	Average	Range	Average	Range
Brown Rice (3)	12	5.6 - 15.4	16	5.2 - 22.5	20	15.8 - 25.4
Germinated rice [a] (1)	38	----	25	----	12	----
White rice (8)	18	0.0 - 29.7	39	0.0 - 82.8	19	13.5 - 33.5
Short-grain (4)	16	0.0 - 29.7	50	7.0 - 82.8	18	13.5 - 22.7
Long-grain (4)	21	15.8 - 29.2	19	0.0 - 46.7	21	14.2 - 33.5
Rice noodles (7)	44	29.0 - 80.4	23	0.0 - 44.0	5	1.0 - 14.4
Group 1 (4)	46	29.0 - 80.4	25	14.8 - 38.9	3	1.1 - 5.5
Group 2 (3)	34	28.6 - 49.1	21	0.0 - 44.0	7	1.0 - 14.4
Rice crackers (7)	29	3.4 - 49.4	32	0.0 - 69.7	11	0.0 - 26.6
Infant foods (4)	50	15.9 - 86.8	10	1.4 - 19.8	14	6.1 - 30.1

[a]: Mean of duplicate measurements in one sample

As shown in table 3, the in vitro solubility of minerals differed among the product categories. The solubility of calcium ranged between 0-87%, with the lowest average (12%) in brown rice and the highest (50%) in infant foods. Iron and zinc solubility ranged between 0-83% and 0-34%, respectively. Both white and brown rice categories showed the highest average solubility of iron and zinc. In both primary and intensively processed products, the average solubility of calcium and iron was much higher than in brown rice. In contrast, germination as well as intensive processing (noodles and crackers) led to a decreased solubility of zinc.

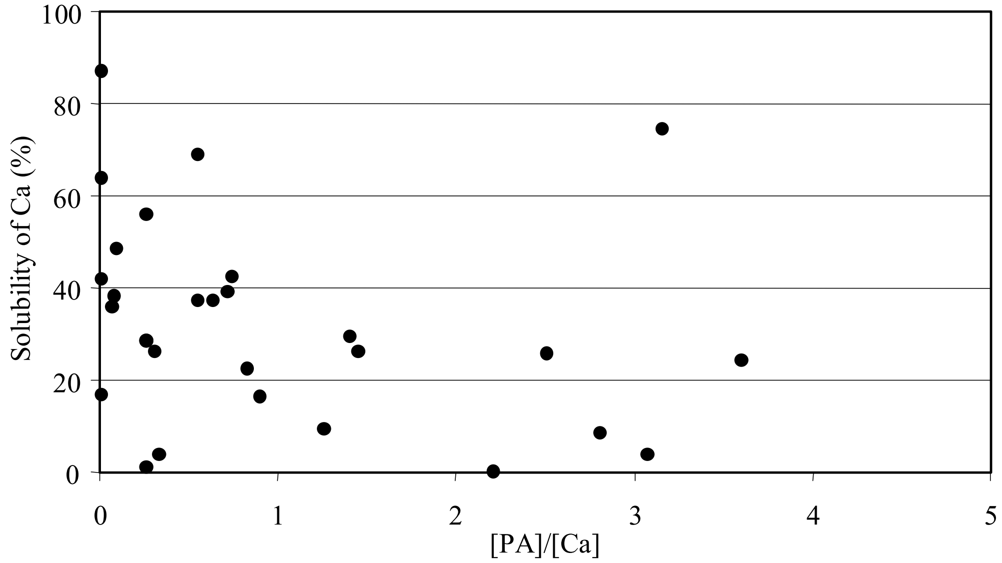


Figure 2: In vitro solubility of calcium in consumer rice products

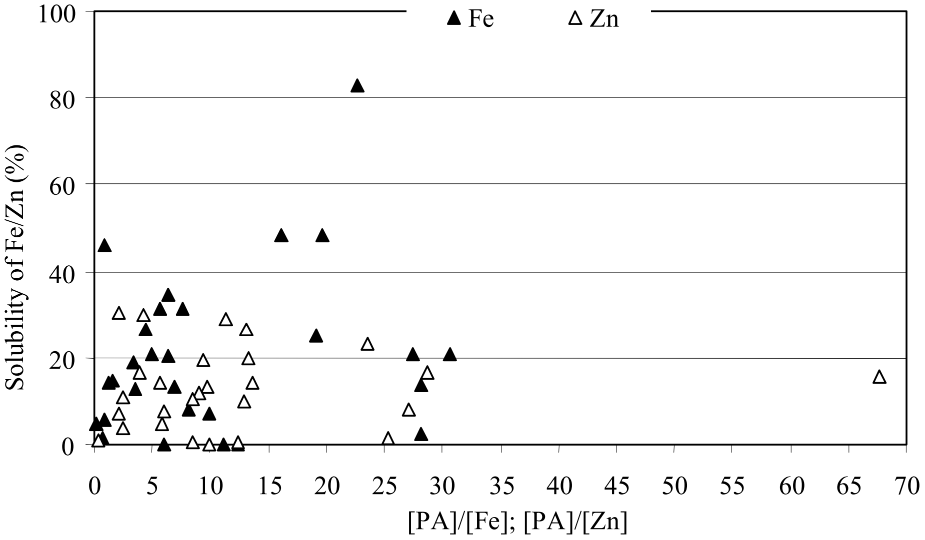


Figure 3: In vitro solubility of iron and zinc in consumer rice products

Figures 2 and 3 show the in vitro solubility of calcium, and iron and zinc, respectively, in relation with the [PA]/[mineral] ratios for each of the samples analysed. Calcium solubility tended to decrease when molar ratios of phytic acid to calcium increased from 0 to 1. Beyond a ratio of 1, most products had a calcium solubility of around 20%. A similar phenomenon was observed in model studies . The iron solubility in about two thirds of the products was lower than 30%, and that of zinc had a narrow range between 6-30%. The solubilities of iron and zinc were not significantly affected by molar ratios of phytic acid to minerals. Figures 2 and 3 indicate that mineral solubility is not exclusively determined by the molar ratio

[PA]/[Fe], but that other factors also play a role. Such factors may include added fortificants and the presence of food matrix components such as dietary fiber and free phosphate.

DISCUSSION

According to Chinese Dietary Reference Intake (DRIs) for adults, the adequate intake (AI) of iron is 10 mg/day and of calcium is 400 mg/day, and the DRI of zinc is 8 mg/day (Chinese Nutrition Society 2006). When calculated on the basis of the per capita consumption of rice and rice products of 238 g/day (Wang 2005) and the average contents of minerals found in the present study, rice-based products supply 11% of calcium AI, 38% of iron AI, and 39% DRI of zinc.

Primary processed rice products cannot be considered as good sources of minerals for several reasons. First, the solubility (as an index for bioavailability) of calcium and iron was very low in brown rice because of a high level of phytic acid. Milling and polishing achieves 90% removal of phytic acid thus increasing the minerals solubility. However, the levels of calcium and iron are also reduced significantly (70-80%) by primary processing. With respect to calcium and iron, germination is a good way to improve their solubility (bioavailability), since both the levels as well as the solubility of calcium and iron increased after germination. Enzymatic degradation of phytic acid and other chelators of minerals during steeping and germination of brown rice contributed to the increase of solubility. On the other hand, for all primary products, white rice is the most important staple food, while brown rice - a good source of zinc - and germinated rice are not widely consumed because their sensory properties are not appreciated by most Chinese consumers (Huang 2004).

Intensively processed rice products are popular with various groups of Chinese consumers, so they also have an impact on the human mineral status. Rice noodles are used as staple foods, especially in Southern China (Lu et al. 2005). The nature of the noodle making process makes noodles an attractive vehicle to enhance minerals intake. During noodle making, rice is soaked and fermented during periods ranging from several hours to 3 days, prior to noodle making. During these preliminary phases, endogenous rice phytase as well as microbial phytases produced by fermentation microbiota reportedly degrade phytic acid (Umeta et al. 2005), which may explain the low levels of phytic acid observed in rice noodles. Differences in noodle making procedures result in variations of product composition (Marfo et al. 1990). Like phytic acid, also the levels of minerals in rice noodles are determined by processing procedures or/and the use of ingredients and equipment. For calcium, the lowest value was caused mainly by the loss of calcium by leaching effects, while the highest levels are probably due to the application of calcium-rich additives. Concerning iron, all noodles had higher iron levels than white rice. We suppose this is related to the use of cast iron processing machines. Neither equipment nor ingredients affected zinc of which the levels are similar to those in white rice. Crackers are mainly consumed in small quantities as a snack and thus will not greatly affect the mineral status. Compared to other products, the infant foods are more important, since they constitute an almost exclusive dietary source of macro- and micronutrients, and because they are consumed in relatively small quantities (Lind et al.

2003). Mineral solubility in e.g. infant foods can be improved by various strategies. Enzymic pre-treatment of white rice e.g. with phytase will significantly improve mineral solubility, as was shown elsewhere with soya bean formulas (Davidsson 2003). Data presented here show an inverse relation between phytic acid levels and solubility of calcium and zinc. The inadequate solubility of iron in some infant foods tested, might be due to the use of an ineffective chemical form of iron used for fortification (Engle-Stone et al. 2005). In addition, interaction of minerals also affects the solubility of calcium, iron and zinc (Fordyce et al. 1987). However, some studies mentioned that even the combination of favourable factors such as reduction of phytic acid, sufficient fortification of iron and addition of ascorbic acid (an enhancer of iron absorption) did not clearly improve iron and zinc status (Lachat et al. 2006; Lind et al. 2003; Mamiro et al. 2004). This suggests that other inhibitory factors such as dietary fiber and the product matrix, may interfere with mineral uptake. Therefore, the ultimate test remains the in vivo measure of uptake.

Promising approaches for the enhancement of bioavailability of minerals in rice products are to increase mineral levels by supplementation or fortification, increase bioavailability through added enhancers or by removal of inhibitors (Davidsson 2003; Gibson et al. 2000) or combinations thereof. Considering the factor 3 variability of iron and zinc levels in rice varieties from growing regions in China (Graham et al. 1999; Liang et al. 2007), there is a potential in selecting crops with maximum mineral and minimum phytic acid levels. The specific localization of minerals and phytic acid also enable an optimised milling procedure resulting in maximum retention of minerals and removal of phytic acid (Liang et al. submitted-c). This would contribute to improved bioavailability of particularly iron, zinc and calcium (Engle-Stone et al. 2005). Fortification with minerals should take into account their interactions, palatability especially for iron compounds, and opportunities for enhancement by ascorbic acid-rich fruit and vegetables (Davidsson 2003; Engle-Stone et al. 2005; Kennedy et al. 2002).

CONCLUSIONS

The diversity of products and processing methods for rice offers opportunities for improvement of mineral bioavailability in rice-consuming regions. From the presented results of contents and solubility of minerals, it was found that primary processed rice products are poor sources of minerals, either because of low bioavailability or because of their low consumer acceptance. Some intensively processed products (except infant foods), e.g. rice noodles, could improve mineral nutrition via the approaches of the use of materials (ingredients) and application of processing methods. Some infant foods need further processing to decrease their levels of PA or other inhibitors, thus increasing mineral bioavailability.

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Chapter 7

General discussion

Rice is important for people in China, not only as a source of energy, but it also plays an important role in the supply of minerals. Unfortunately, rice appears to be a poor source of minerals, because it also contains high levels of phytate. The studies in this thesis mainly focused on improving bioavailability of minerals, particularly iron and zinc, in rice and rice products, by removing or degrading phytate. As an indicator of bioavailability, we measured the in vitro solubility of minerals after enzymatic digestion of the food samples. Studies addressed the effect of rice varieties and growing environment, the effect of dry milling, the effects of wet processing, and the composition of consumer rice products. Results are discussed in this chapter, followed by the advantages and shortcomings of these approaches. Finally, recommendations for policy, implementation and further research are made.

SUMMARY OF RESULTS

The main results of the thesis are summarized and presented in table 1.

Table 1: Summary of results of the thesis

Research questions proposed	**Main results obtained**
Variation of minerals and inhibitors in rice from China: • There are many rice varieties in China. What is the variation of levels of minerals (iron and zinc) and phytic acid in rice in China?	***Variation of substances analysed:*** Iron levels averaged 18 mg kg^{-1} with a big variation (9-45 mg kg^{-1}). Average zinc contents were 23 mg kg^{-1} with a moderate variability (13-39 mg kg^{-1}). Phytic acid average content was 10 g kg^{-1}, with the narrowest range (7.2-11.9 g kg^{-1}). With the samples used, it was not possible to draw conclusions on varietal effects.
• Growing conditions differ quite a lot in China. Do growing regions affect levels of minerals and inhibitors in rice?	***Effect of environment:*** Iron contents were significantly ($p <0.05$) higher in the rice cultivated in Jiangxi, and lower in Fujian, Jiangsu and Jilin. The level of zinc content in the rice from different growing regions was significantly different ($p <0.05$). Rice grown in Jilin significantly had lower phytic acid levels.
• What are the levels of the bioavailability of brown rice without processing? (Chap. 2)	***Prediction of bioavailability of minerals in brown rice:*** Molar ratios of PA to Fe and Zn ranged from 15-105 and 27-67, respectively. The mineral bioavailability of Chinese brown rice varieties is estimated to be lower than 4%.

Effect of dry milling: • White rice is the major consumption product in China. To what extent will minerals and phytic acid be lost during dry milling?	***Loss of minerals and phytic acid during milling:*** At mass losses of about 5%, phytic acid levels in milled rice are only 11-28% (depending on variety) lower than in brown rice. While at the same milling rate, zinc levels in all varieties of milled rice were still at the same level as in brown rice. However, an almost complete removal of phytate could only be achieved after removing 20-25% of initial rice mass.
• Are minerals and phytic acid located at the same place in rice kernels?	***Distribution of minerals and phytic acid in rice kernel:*** The concentration of phosphorus decreased from the surface region inward; about 25% of the phytic acid is located in the perisperm of the kernel. The highest concentration of phytic acid was observed at the interface of embryo and perisperm. All rice varieties studied had the highest concentration of zinc in the embryo whereas it was relatively evenly distributed in the other parts of the grain.
• Is it possible to remove inhibitors (phytic acid), but retain minerals during dry milling? (Chap. 3)	***Possibility to improve bioavailability of minerals by dry milling:*** Theoretically, different distribution of minerals (zinc) and phytic acid gives possibilities to obtain milled rice with minimum loss of weight and minerals, at a maximum removal of phytic acid. However, from an economic point of view this is not feasible.
Effect of wet processing: • Approaches of soaking, germination, fermentation were reportedly effective to decrease phytic acid in other cereals. Do they also have this effect in rice?	***Effect of soaking and phytase treatment on phytic acid in different fractions of rice:*** For brown rice, the most effective way of phytic acid reduction is grinding the kernel to small particle-size (flour) first, followed by phytase treatment. In white rice, both extended soaking or phytase treatment were adequate. For rice bran, phytase treatment was the most effective. When considering changes in different rice fractions, soaking with commercial phytase at optimum conditions, or in acidic buffer after grinding is more effective to decrease phytic acid than soaking in water.
• Of these methods, which one is the most	***Comparison of wet processes for their effectiveness:*** With regard to the reduction of phytic acid, accelerated

effective in reduction of phytic acid in rice?	fermentation was the most effective approach (56-96%), followed by soaking (42-59%). Reduction of phytic acid increased with germination time (4-60%). Steeping had the lowest effect.
- How does wet processing enhance the bioavailability of minerals? (Chap. 4, 5)	***Bioavailability of minerals (zinc):*** Soaking after preheating significantly increases its solubility because of loss of total zinc during soaking, while steeping (without preheating) decreased it significantly ($P<0.05$). Fermentation and germination did not have significant effect on solubility of zinc.
<u>Rice products:</u> - There are many types of consumer rice products in China. What is the bioavailability of minerals in these rice products? (Chap. 6)	***Present levels and bioavailability of minerals and phytic acid in consumer rice products:*** Brown rice had much higher calcium, iron and phytic acid levels than white rice. Primary processed rice products had high molar ratios of phytic acid to minerals. [PA]/[Ca] <5, [PA]/[Fe] and [PA]/[Zn] ranged from 5-74 with most values between 20-30. Rice noodles had the lowest phytic acid and the broadest range of mineral contents; in particular, noodles had the highest levels and solubility of calcium. In rice crackers, the levels and solubility of minerals were similar as that in rice noodles. In infant foods, the highest levels of minerals were observed because these products have been fortified with added minerals. In intensively processed rice products, the [PA]/[mineral] molar ratios were generally lower. They are ranged at 0.0-3.1, 0.2-21 and 0.3-27 for molar ratios of [PA]/[Ca], [PA]/[Fe] and [PA]/[Zn] respectively.

APPROACHES TO IMPROVE BIOAVAILABILITY OF IRON AND ZINC IN RICE

Varieties and agricultural practice

Rice is widely cultivated in China and plays an important role in nutrition of minerals in China (Ma 2007; Zhou 1993). Variable environmental conditions (i.e. temperature, rainfall, sunshine, soil), and farming practice in growing regions lead to the use of diverse cultivars of rice, even in the same region (Zhou 1993). Data in chapter 2 indicated that nation-wide, the highest concentration of iron was about 5 times than the lowest; and that the highest zinc concentration was 3 times higher than the lowest. Phytic acid levels were relatively stable (highest ~1.5 times higher than lowest). Comparison of mineral contents and phytic acid levels of rice harvested in different years (chapters 2, 3 and 4) showed that, in addition to varietal influence, agricultural practice and agronomic conditions (soil, climate) might have considerable effect on the chemical composition of rice.

These results suggest that:

1) With the present varieties used in the different growing regions, levels of minerals in brown rice could be increased by changing agricultural practice. For example, levels of iron and zinc in irrigated rice were more than 20% higher than in aerobic rice from the same growing region in the same harvest year (chapter 2). Studies on wheat indicated that mineral levels in seeds could be increased by pre-harvest application of minerals (Yilmaz et al. 1997);

2) The diversity of environmental conditions in China offers the opportunity to select proper rice varieties for certain regions, which combine high yields with relatively high mineral levels and lower levels of inhibitors such as phytic acid. This agronomic optimization is time and labor intensive, and it might take a long time to find the suitable varieties for certain regions. Nowadays, rice breeding in China is mainly focused on yield and stress tolerance (Yu et al. 2001). We advise that more attention is paid to optimizing minerals and phytic acid contents;

3) Breeding rice varieties with either traditional or modern methods offers also an effective way to improve mineral nutrition in rice. Success of application of hybrid rice in South China is a positive model for developing high bioavailability rice varieties in China. The high levels of minerals and low levels of phytic acid observed with cultivated rice offer opportunities to breed new varieties with higher minerals and lower inhibitors by traditional breeding technologies. Such varieties could also help to increase the bioavailability of phytase treated materials, since lower phytic acid levels release less phosphate upon phytase treatment, thus causing less formation of insoluble mineral phosphates. Modern biotechnology has also been used in breeding for improved bioavailability of minerals (particularly iron and zinc). Lucca *et al.* bred a new variety of rice containing twofold higher iron levels, and rich in phytase (phytase activity sufficient to completely degrade phytic acid) and containing a cysteine-peptide (a major enhancer of iron) by means of genetic engineering. They introduced a ferritin gene from *Phaseolus vulgaris*, a thermo-tolerant phytase gene from *Aspergillus fumigatus* and over-expressed the endogenous cysteine-rich metallothionein-like protein in rice (Lucca et al. 2001; Lucca et al. 2002). Other researchers also proposed to introduce a fungal phytase gene (Gibson et al. 2000; Grotz and Guerinot 2002; Welch and Graham 2004; Welch et al. 2000). However, the application of genetic engineering for food purposes is still debated. At present, the most reliable and feasible option is to optimize agronomic practice, followed by conventional breeding; in time more experience has been gained with transgenic rice, which would warrant its introduction into agricultural farm practice.

Implication of dry milling

Dry milling is a very important procedure in rice processing, since white rice is the most popular rice product in China. In order to obtain attractive appearance and edibility of milled rice, traditionally about 10-15% of outer layer of brown rice has to be removed by milling and polishing (CHISA 2002; Ruan 2005). We found that removal of 98-99% of phytic acid by dry milling is achieved only when more than 20% of the outer parts have been removed (chapter 3). That means:

1) For nutritional purposes: consumers will mainly get starch, some protein and only a small quantity of minerals (e.g. molybdenum and zinc) from rice, since many important nutrients, like minerals (iron), vitamins and fibers are lost in the rice bran fraction (Bajaj et al. 1989; Doesthale et al. 1979; Itani et al. 2002; Kennedy et al. 2002; Resurreccion et al. 1979; Tabekhia and Luh 1979). That is, even if the minerals have a high bioavailability, this option is not helpful in improving mineral status, because of the low proportion of minerals in the endosperm;

2) For economic purposes: The most important effect of dry milling is to obtain light-colored and edible white rice. All removed mass goes into rice bran, which is considered as waste and is mainly used in animal feed. Higher milling rates mean even more waste, or higher cost for the rice producing industry. Furthermore, higher milling rates will lead to a higher extent of breakage, which will even decrease the yield of white rice further.

So, both from nutritional as well as economic viewpoints, improving bioavailability of minerals in rice by increasing dry milling rates does not seem feasible.

Implication of wet processing

Wet processing includes several different methods, such as soaking, germination, fermentation and phytase treatment. Soaking in liquid (water) for some time for water/liquid absorption is a first essential step.

Soaking is an important preparation procedure both at home and at industrial scale. Results in this thesis indicated that soaking could reduce phytic acid in rice fractions (chapter 4). Germination used in rice processing was developed in recent years for the improvement of functionality, especially enrichment of brown rice with GABA (gamma amino butyric acid) (Huang 2004). Studies on phytase activity (data are not shown in this thesis) and bioavailability of minerals during germination indicated that besides its effect on GABA, germination also could improve bioavailability of calcium and iron with about 25% and 10%. However, germination did not have a significant effect on bioavailability of zinc (chapters 5 and 6). Natural lactic acid fermentation is an important procedure to soften and prepare rice for noodle making (Lu et al. 2005). Results in this thesis showed that fermentation (as in rice noodle making) could effectively improve the bioavailability of calcium and iron, but not of zinc (chapters 5 and 6). Commercial phytase is widely used in the feed industry to decrease phytate and thus to improve availability of phosphorus. In this thesis, application of phytase was carried out to reduce phytic acid in rice fractions. This approach proved to be the most effective way to decrease phytic acid levels (chapter 5).

Further studies will be needed to study the effect of phytase on the bioavailability of minerals in rice.

Some issues regarding the in vitro solubility of minerals need further study. We observed that in spite of degradation of phytic acid, levels of soluble minerals did not always increase as expected. Whereas this may be caused by the complex-forming ability of residual phytic acid, other factors may play a role as well. If phytic acid is degraded, phosphate anions will be released. Certain mineral phosphate salts have a low solubility, hence it may happen that minerals change from one insoluble complex (phytate) into the other (phosphate). This uncertainty also indicates the importance of in vivo trials to validate in vitro procedures.

From our wet processing experiments, we conclude that wet processing methods are promising approaches to improve the bioavailability of minerals in rice. Further research is needed to optimize processing for sensory quality and improved mineral bioavailability in final products.

RECOMMENDATIONS FOR POLICY AND FURTHER RESEARCH

Information and education of consumers about the importance of minerals in the diet

Mineral (especial iron and zinc) deficiency is a serious problem in China (Ma 2007; Wang 2005). However, only researchers and educated consumers are aware of this and obviously this is inadequate to improve the mineral status of the total population. On the short term, supplementation and fortification of foods might be effective to reach average consumers. But on the long term, interventions based on biofortification and diversification of the diet are more likely to succeed (Ma 2007).

In order to achieve the objective and improve mineral status in the long term, changing the consumption habits of the Chinese consumer may be facilitated by information, keeping in mind that it is not easy to influence patterns of dietary choice. Chinese people do not easily accept new products with unusual sensory properties, even if they would be more nutritious. Germinated rice is an illustrative case. Because of its nutritional value, it is very popular in Japan (Huang 2004). A Chinese company in north east China also started to produce germinated rice in 2003. Investigation with students of the College of Food Science and Nutritional Engineering at China Agricultural University showed that germinated rice is not accepted because of its perceived poor sensory properties. A survey with supermarkets showed the same result. We conclude that consumers in China pay more attention to sensory acceptability than to nutritional value. Maybe, this could be changed by long-term information and consumer education, especially those low-educated consumers living in rural areas, about the importance of nutrients and recommended diet composition. Alternatively, an effort could be made to improve the sensory quality of these products.

Fortification minerals in rice products

Fortification of staple foods and condiments is widely used to combat mineral deficiency (Mannar and Gallego 2002), and it might be a feasible, cost-effective and sustainable way to alleviate iron and zinc deficiency in China (Ma 2007). However, with the exception of fortification of salt with iodine, fortification of other minerals just started on a trial basis in recent years in China. For example, in a county of south west China, where there is a big problem of iron deficiency, intervention trials with iron fortification of soy sauce using EDTA-iron were carried out 4 years ago. The intervention study is still running and results will be published shortly (Chinese Center for Diseases Control and Prevention 2007). A study on the *in vitro* solubility of minerals in infant foods indicated that fortification can increase status of minerals, although solubility of minerals is still at low level (chapter 6). From the results of this thesis, it was found that >70% of iron and almost all rice embryos, which have the highest density of zinc, goes into the bran. At the same time, high levels phytic acid (~70%) also go into the bran (chapter 3). Whereas it is common practice to separate the embryo from wheat and maize, we expect that this is more difficult with rice. However, the rice embryo contains high levels of valuable nutrients and an effort should be made to develop processes towards its extraction. It may also be attractive both for industry and consumers, if rice bran could be added back to enrich rice products after a treatment to remove phytic acid and other inhibitors of mineral uptake. Effective treatments might be application of enzymes, or fermentative processing. From the results of this thesis, rice noodles could be a good carrier for fortification. Further research will be required to measure and understand the effects of addition of rice bran or fractions thereof, on sensory aspects of noodles.

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Summary

Rice plays an important role in the Chinese diet as two thirds of the population consume rice as their staple food. Rice is not only a source of energy and protein, but also of essential minerals. At present, deficiencies of minerals, especially iron and zinc, are prevalent in China, notwithstanding that the consumption of iron is about twice the recommended level. The main reason for this discrepancy is the low bioavailability of minerals from plant-derived foods which predominate the diet in most developing countries. The knowledge about bioavailability of minerals, and dietary factors that enhance or inhibit the uptake of minerals from food, is still inadequate. Post-harvest processing of rice includes dehusking to give brown rice, and milling and polishing to obtain white rice. Other rice-derived products such as germinated rice, rice noodles etc. require more complex, wet processes. This thesis aims at contributing such knowledge about the occurrence of iron and zinc and the main inhibitor phytic acid, in rice from China, as affected by food processing, such as dry milling and wet processing.

At first, a selection was made of 56 major rice varieties grown in six rice growing regions of China (Chapter 2). These varieties were characterized by kernel dimensions, milling quality and their contents of iron, zinc and phytic acid (PA). The variation of iron levels was considerable (9-45 mg kg^{-1}) and was not related to PA content or kernel shape, but iron levels ranged from low (14.2 mg kg^{-1}) to high (25.2 mg kg^{-1}) in distinct growing locations. Zinc levels showed less variability (13-39 mg kg^{-1}) than iron, and PA contents were even more narrowly ranged (7.2-11.9 g kg^{-1}). Zinc contents were positively correlated ($R^2 = 0.5$; $p<0.01$) with phytic acid, and showed also a relation with growing region and kernel shape. High molar ratios of phytic acid to iron and zinc are correlated with poor availability of the minerals. In brown rice [PA]/[Fe] ranged from 15-105 and [PA]/[Zn] from 27-67, respectively. According to established predictive models, this indicates a very low bioavailability (< 4%) so that improvements are required.

Based on the study mentioned above, three varieties with high levels of PA and minerals, but of different kernel shapes were selected for processing studies. Chapter 3 deals with the effects of dry milling of brown rice. Experimental abrasive milling and X-ray fluorescent microscopic imaging were used to analyse and predict the effect of dry milling on losses of rice mass, minerals and PA. As expected, the milling characteristics were different according to kernel shape and variety. Levels of PA, as well as iron and zinc decreased with prolonged milling. Phytic acid was removed earlier than zinc. X-ray fluorescent images indicated that the highest concentrations of phosphorus (indicator for PA) and zinc were located at the interface of embryo and perisperm, and within the embryo, respectively. This knowledge of the spatial distribution of valuable components in the kernel offers opportunities for optimization of milling procedures for maximum PA removal, at minimum mineral losses and yield loss.

The impact of wet processing operations such as soaking, germination, phytase treatment and fermentation were described in Chapters 4 and 5.

In order to evaluate the effects of soaking and phytase treatment, studies were designed to distinguish the effects of added, versus endogenous phytase that is activated during soaking. In order to inactivate the endogenous phytase, rice samples were preheated prior to other process treatments. Studying the degradation of PA from different rice grades (brown rice kernels, brown rice flour, white rice kernels, white rice flour, and rice bran), it was observed that removal of PA from brown rice was facilitated by grinding to small particle sizes (flour) first, followed by phytase treatment. For the removal of PA from white rice, both soaking with long-duration or phytase treatment were successful. From rice bran, PA could only be removed adequately by phytase treatment. A comparison of wet processing on the bioavailability (estimated as in vitro solubility) of minerals indicated that soaking of preheated rice significantly increased zinc solubility due to loss of total zinc, while steeping as a first step of germination decreased zinc solubility ($P<0.05$). Fermentation and germination did not have a significant effect on the solubility of zinc.

In Chapter 6, several consumer rice products as sold in Chinese supermarkets, were analysed for their minerals and PA content, and the in vitro solubility of the minerals was measured. Among six categories of rice products, brown rice had much higher calcium, iron and phytic acid levels than white rice. Primary processed rice products (white rice mainly) had high molar ratios of phytic acid to minerals, namely [PA]/[Ca] < 5, and [PA]/[Fe] and [PA]/[Zn] ranging from 5–74, mostly between 20-30. Of the intensively processed rice products (noodles, crackers, germinated rice, infant food), rice noodles had the lowest phytic acid and the broadest range of mineral contents and in particular the highest levels and solubility of calcium. In rice crackers, the levels and solubility of minerals were similar to those in rice noodles. In infant foods, the highest levels of minerals were observed. This has to be attributed to mineral fortification. Although in intensively processed rice products, the [PA]/[mineral] molar ratios were generally lower, the data indicate that at present, both primary and intensively processed rice-based products cannot be considered as good dietary sources of minerals. Improvements may be expected from a further increase of mineral levels, e.g. by mineral fortification, and dephytinization, or – preferably – combinations of both.

In the discussion, several approaches to improve mineral bioavailability are mentioned and compared. Selection of existing or new rice varieties for use in specific growing regions, which are compatible with local agricultural practice, is advised to be a sustainable starting position for primary production. Post-harvest processing by dry milling is a wasteful practice which leads to considerable losses of valuable food material before sufficient phytic acid is removed. Wet processing approaches hold promise because they combine improved mineral availability with consumer acceptability and economic feasibility. But unfortunately, these have not yet resulted in sensory acceptable rice products. We therefore proposed to treat bran fraction and convert it to a source of bioavailable nutrients that can be added to rice products or used in condiments.

On the short term, the mineral status of rice products could be improved by dephytinization and fortification with added minerals. On the long term, new rice varieties, obtained by either conventional or biotechnological techniques, that combine high mineral and low phytic acid contents, may be introduced. But in the end, the choice of the consumer is crucial. The general public needs to be informed about the importance of a healthy lifestyle and good nutrition, in order to arrive at a dietary diversification for the supply of macro- and micronutrients.

Samenvatting

Rijst wordt door tweederde van de Chinese bevolking als hoofdvoedsel gegeten en speelt daarmee een belangrijke rol in het voedingspatroon. Behalve energie en eiwit levert rijst ook essentiële mineralen. Tegenwoordig komen mineralentekorten, vooral van ijzer en zink, veelvuldig in China voor, ondanks een mineraleninname per hoofd van de bevolking die het dubbele bedraagt van de aanbevolen hoeveelheid. De belangrijkste reden voor deze tegenstelling is de geringe opname (wegens geringe biobeschikbaarheid) van mineralen uit plantaardig voedsel, waaruit het voedselpakket in ontwikkelingslanden overwegend bestaat. De kennis over de biobeschikbaarheid van mineralen, en de voedselgerelateerde factoren die de opname van mineralen bevorderen of remmen, vertoont nog leemtes. Na de oogst wordt rijst verwerkt door pellen waarmee bruine of zilvervliesrijst wordt verkregen, en vervolgens kan door slijpen en polijsten witte rijst worden geproduceerd. Andere producten worden van rijst gemaakt zoals gekiemde rijst en noedels; hiervoor worden gecompliceerde natte processen gebruikt. Dit proefschrift levert een kennisbijdrage omtrent de prevalentie in rijst uit China, van ijzer, zink en de opnameremmer fytinezuur, en de mate waarin deze stoffen worden beïnvloed door verwerking zoals slijpen en natte processen.

Een eerste studie werd gemaakt van 56 belangrijke rijst variëteiten verbouwd in zes rijstproducerende gebieden van China (Hoofdstuk 2). De eigenschappen van deze variëteiten zoals hun korreleigenschappen, gedrag bij slijpen en hun ijzer, zink en fytinezuur (FZ) gehalte, werden onderzocht. Er werd een flinke spreiding in ijzergehalte geconstateerd (9-45 mg kg^{-1}) die niet gecorreleerd was aan FZ gehalte of korrelvorm, maar wel uiteenliep van laag naar hoog in verschillende kweekgebieden. Zink vertoonde minder spreiding (13-39 mg kg^{-1}) dan ijzer, en FZ spreiding was zelfs nog minder (7.2-11.9 g kg^{-1}). Zinkgehalten waren positief gecorreleerd ($R^2 = 0.5$; $p < 0.01$) aan FZ, en ook gerelateerd aan kweekgebied en korrelvorm. Hoge molaire quotiënten van FZ/Fe en FZ/Zn worden geacht te leiden tot verminderde biobeschikbaarheid van deze mineralen. In zilvervliesrijst liep de FZ/Fe uiteen van 15 tot 105 en FZ/Zn van 27-67. Volgens eerder gepubliceerde modellen voorspellen deze quotiënten een zeer geringe (<4%) biobeschikbaarheid, hetgeen vraagt om verbetering.

Op basis van de eerstgenoemde studie werden drie variëteiten met hoog FZ en mineralengehalte, en van verschillende korrelvorm, geselecteerd voor verdere experimenten. Hoofdstuk 3 behandelt de effecten van droge bewerking (slijpen) op zilvervliesrijst. Slijpproeven en Röntgen fluorescentiemicroscopie werden gecombineerd om de invloed van slijpen op verlies van materiaal, mineralen en FZ te meten en te voorspellen. Zoals verwacht was het gedrag bij slijpen afhankelijk van de korrelvorm en de variëteit. Langdurig slijpen leidde tot toenemende verliezen van opbrengst, FZ, ijzer en zink. Fytinezuur werd sneller verwijderd dan zink. Röntgen fluorescentie beelden toonden dat de hoogste concentraties fosfor (een indicator voor FZ) en zink zich bevinden in de kiem, en op de scheiding tussen kiem en perisperm. De kennis over de ruimtelijke verspreiding van waardevolle componenten in de korrel biedt mogelijkheden voor verbetering van slijpmethoden om een maximale verwijdering van FZ en minimale verliezen van mineralen en materiaal te verkrijgen.

De invloed van natte processen zoals weken, kiemen, fytase enzymbehandeling en fermentatie, wordt besproken in hoofdstukken 4 en 5.

Teneinde de invloed van weken en fytase enzymbehandeling afzonderlijk te kunnen meten werden proeven ontwikkeld om toegevoegd fytase van het plant-eigen (endogeen) fytase te kunnen onderscheiden; het laatstgenoemde enzym wordt geactiveerd tijdens het kiemen. Endogeen fytase werd hiertoe geïnactiveerd d.m.v. voorverhitting van rijstmonsters. Er werd geconstateerd dat in verschillende rijstproducten (hele zilvervliesrijst, gemalen zilvervliesrijst, hele witte rijst, gemalen witte rijst, en rijstzemelen) de verwijdering van FZ uit zilvervliesrijst werd bevorderd door malen, gevolgd door behandeling met fytase. Om FZ uit hele witte rijst te verwijderen waren langdurig weken, of fytasebehandeling het meest effectief. Verwijderen van FZ uit rijstzemelen lukte het beste d.m.v. fytasebehandeling. De biobeschikbaarheid (geschat als in vitro oplosbaarheid) van zink was groot in voorverhitte hele witte rijst, gezien het grote verlies van zink dat in het weekwater oploste. Inweken van onverhitte korrels als eerste stap van het kiemen veroorzaakte een significante ($P<0.05$) verlaging van zink oplosbaarheid. Fermentatie en kieming hadden geen significante invloed op de zinkoplosbaarheid.

Hoofdstuk 6 beschrijft een analyse van een aantal rijst consumentenprodukten die in de supermarkt worden verkocht. Gemeten werden mineralen- en FZ-gehalte en in vitro oplosbaarheid van mineralen. Van zes groepen rijstprodukten had zilvervliesrijst veel hogere calcium, ijzer en FZ gehalten dan witte rijst. Basisproducten, vnl. witte rijst, hadden hogere molaire quotiënten van FZ/mineralen, namelijk FZ/Ca < 5, FZ/Fe 5-74, en FZ/Zn 20-30. Van de rijstproducten (noedels, koeken, gekiemde rijst en kindervoeding) hadden noedels het laagste FZ gehalte en de grootste spreiding in mineralengehalten, waaronder de hoogste gehalten en oplosbaarheid van calcium. Rijstkoeken hadden dezelfde mineralengehalten en spreiding als noedels. In kindervoeding waren de mineralengehalten hoger omdat deze waren verrijkt. Hoewel van rijstproducten de FZ/mineralen quotiënten lager (gunstiger) waren dan van basisproducten, kan van beide groepen worden geconstateerd dat het toch geen goede bronnen van opneembare mineralen zijn. Een verbetering hierin zou kunnen worden verkregen door verrijking met toegevoegde mineralen, en vérgaande fytinezuurverwijdering, of bij voorkeur een combinatie hiervan.

In de discussie worden verschillende mogelijkheden vergeleken om de mineralen beschikbaarheid in rijstproducten te verbeteren. Selectie van bestaande of nieuwe rijstvariëteiten toegespitst op gebruik in specifieke kweekgebieden, en passend in de lokale landbouwpraktijk, is een duurzame keuze voor primaire productie. Verwerking na de oogst door droge slijpmethoden is een verkwistende aanpak die tot grote opbrengstverliezen leidt voordat men fytinezuur afdoende heeft kunnen verwijderen. Natte processen zijn veelbelovend omdat hiermee verbeterde mineralenbeschikbaarheid samengaat met goede acceptatie door de consument, en aan economische haalbaarheid.

Op de korte termijn kan de mineralenbeschikbaarheid in rijstproducten worden verbeterd door volledige fytinezuurverwijdering en mineralentoevoeging. Op de langere termijn dient gestreefd te worden naar nieuwe rijstvariëteiten met hoge mineralen- en lage fytinezuurgehalten. Echter, uiteindelijk bepaalt de consument wat er gegeten wordt. Een goede publieksvoorlichting over gezonde leef- en voedingsgewoonten is van belang om tot een gevarieerd menu te komen dat voorziet in de noodzakelijke macro- en micronutriënten.

概要

水稻在中国居民的膳食中占据非常重要的地位，三分之二的居民以它作为主食。水稻是居民膳食中能量和蛋白质的重要来源，同时也提供一些重要的矿物质。目前，中国居民的矿物质缺乏还很普遍，特别是铁和锌的缺乏。铁缺乏问题并没有因为铁摄入水平的明显提高（中国居民铁实际摄入量为建议摄入量的 2 倍多）而有所改善。导致这种现象的主要原因是中国居民膳食铁的主要来源是植物类食物。相对于动物来源的铁来讲，植物来源的铁生物利用率很低。关于食物中矿物质的生物利用率情况，以及膳食中哪些因素可以促进、哪些因素会抑制矿物质的吸收，目前还没有一个明确的研究结论。

为了获得良好的食用品质，稻谷从田间收获后要经过包括砻谷去除稻壳，获得糙米，和糙米再经过碾米抛光获得精白米（大米）两个阶段。中国市场上最主要的稻谷制品是精白米，除此之外，还有其它的大米制品，如发芽米、米饼、米粉等。这些稻谷制品分别经过了相对简单的干法加工和比较复杂的湿法加工。本论文主要研究了产于中国的水稻中铁、锌及它们的抗营养因子——植酸的含量及分布情况，并分析了不同加工方法（包括干法和湿法）对植酸及矿物质的生物利用率的影响。

首先，收集了 56 种产于中国六个主要水稻产区主栽品种，并对这些水稻的籽粒特征、碾磨特性、铁、锌和植酸含量分别进行了研究（第二章）。结果显示，产于中国的水稻糙米铁含量的变化范围最大（9-45 mg kg^{-1}）；锌含量的变化范围比铁窄，为 13-39 mg kg^{-1}；植酸含量的变化范围最窄，为 7.2-11.9 g kg^{-1}。铁含量同植酸含量没有相关性，却受到种植区域的影响，不同区域的平均低值和高值分别为 14.2 mg kg^{-1}和 25.2 mg kg^{-1}。锌含量同植酸含量成正比（$R^2 = 0.5; p<0.01$），同时锌的含量同种植区域和籽粒形状有一定的相关性。糙米中植酸与铁的摩尔比为 15-105，与锌的摩尔比为 27-67。这样的植酸与矿物质摩尔比，如果采用现有的数学模型预测，糙米铁和锌的生物利用率仅为 4%左右，非常有必要提高。

基于第二章的研究结果，进一步选取了三种籽粒形状不同、植酸和矿物质含量较高的稻谷品种用于干法加工效果的研究（第三章）。论文采用碾磨法和 X-射线荧光显微法相结合的研究手段，对糙米碾磨特性和碾磨过程造成的矿物质和植酸损失情况进行了系统分析。碾磨法结果显示，不同品种和籽粒形状的糙米，碾磨特性不同；植酸和矿物质的含量随着碾磨时间的延长降低，植酸的损失速度比锌快。X-射线荧光显微法的结果显示，磷（用于指示植酸）含量最高的位置是糙米籽粒的上胚乳和胚芽的交界处；锌含量最高的位置是籽粒胚芽。不同营养成分在糙米籽粒中的不均匀分布情况，为通过优化碾磨时间和碾磨方法，尽可能多地去除植酸，同时减少矿物质和干物质损失提供了可能。

论文第四章和第五章主要研究了湿法加工方法，包括浸泡、发芽、植酸酶处理和发酵，对植酸和矿物质含量、及矿物质溶出率的影响。

为了研究浸泡降低样品中植酸含量的真正原因，本论文在浸泡处理之前对糙米加热灭酶处理，并设计了在浸泡过程中加入商用植酸酶（微生物来源）的实验。对糙米的不同部分（包括糙米籽粒、糙米粉、精白米、精白米粉和米糠）的研究结果表明：对糙米而言，最佳的降低植酸含量的方法是先将籽粒粉碎微细化，然后进行植酸酶处理；对精白米，长时间的浸泡或者短时间植酸酶处理，都可以达到有效降低植酸含量的目的；对

于米糠，只有经过合理的植酸酶处理才可以去除其高含量的植酸。对照研究不同湿法加工方法的效果，浸泡经过湿热处理的糙米，可以显著提高糙米的锌溶出率（主要是由于浸泡后的样品中的总的锌含量下降造成的），而发芽过程的浸泡处理则会降低锌的溶出率（$P<0.05$）；发酵和发芽对处理样品的锌的溶出率没有显著影响。

论文第六章主要研究了收集于北京市场上的一些大米制品的矿物质含量、植酸含量及矿物质的溶出率。在所收集的六类大米制品中，糙米中钙、铁、植酸的含量明显高于精白米的含量。初级加工米制品（保持有米粒形状的产品，包括糙米、精白米和发芽糙米）的植酸和矿物质的摩尔比较高，分别为$[PA]/[Ca] < 5$，$[PA]/[Fe]$及$[PA]/[Zn]$的摩尔比值范围为 5-74，大多数样品的比值在 20-30 的范围内。在所有深加工米制品（看不出大米的形状的产品，包括：米粉、米饼、婴幼儿米粉）中，米粉的植酸含量最低，矿物质含量范围最宽，特别是米粉制品表现出最高的钙的溶出率；米饼的矿物质含量和溶出率同米粉相类似；婴幼儿米粉的矿物质含量最高，这主要是由矿物质强化造成的。深加工品的植酸与矿物质的摩尔比总体比较低。进一步的研究结果显示，在现有条件下，无论是初级加工品，还是深加工品，大米制品都不是好的膳食矿物质来源。这个问题需要通过增加制品中矿物质的含量（例如矿物质强化），或进一步降解制品中的植酸，或者两种方法结合使用而得到改善。

论文的讨论部分，提出了几种可用于改善大米制品的矿物质的生物利用率的方法，并对这些方法进行了一定的分析。对现有的和新发现的稻谷品种进行选择，用于不同的种植区域，同时结合考虑农业操作，是改善稻谷矿物质和植酸含量状况的可持续进行的战略；而通过优化糙米碾磨工艺改善矿物质营养的意义相对比较小，因为伴随着碾磨过程中绝大部分植酸的去除，稻谷的出米率也迅速降低，同时精白米的营养物质也会大量流失，因此无论对稻谷加工企业还是消费者都不经济；湿法加工是比较有应用前景的处理方法，这些方法在改善矿物质营养状况的同时，也可以从一定程度上提高米制品的食味和感官品质。

简言之，米制品的矿物质营养状况可以通过彻底地降解植酸和强化矿物质含量而得到提高。从长远的眼光来看，只有采用传统的和现代生物技术的手段，培育出高矿物质、低植酸的含量的稻谷品种，才可以从根本上解决问题。但是，关于矿物质营养状况问题，最终还是要落到消费者身上，因为只有他们本身的作用才是最关键的。从事营养研究的人员和相关的政府部门需要进行宣传和教育，让人们了解健康的生命和良好营养状况的重要性，从而通过膳食多样化改善常量营养素和微量营养素的营养状况。

Curriculum Vitae

Jianfen Liang was born on September 26th, 1970, in Pingyao, Shanxi Province, China. After primary, secondary and high school in Taigu (Shanxi, China), she was enrolled by Beijing Agricultural Engineering University (presently China Agricultural University) in 1989. She majored in Food Science and Engineering during 1989-1993, and obtained her Bachelor's Degree in July, 1993. From 1993 to 1997, she worked as a researcher in the Institute of Food Engineering at China Agricultural University. She studied for her Master's Degree from 1997-1999 at the College of Food Science and Nutritional Engineering of China Agricultural University and became a teacher there. In October 2002, she obtained a PhD research grant from the Wageningen University through the Interdisciplinary Research and Education Fund (INREF), to undertake a PhD research at the Laboratory of Food Microbiology, Department of Agrotechnology and Food Sciences, Wageningen University and Research Centre, The Netherlands.

List of publications

Jiang H, Ji B, Liang J, Zhou F, Yang Z, Zhang G (2006a) Changes of contents and antioxidant activities of polyphenols during fruit development of four apple cultivars. European Food Research Technology: on-line DOI 10.1007/s00217-00006-00262-00218

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Liang J, Zhang X, Yi J, Li T (2006b) Changes of micro-organisms in brown rice soaking and germination. Food Science and Technology 31: 130-132

Overview of completed training activities

Discipline specific activities:	Credit points
Courses:	
VLAG course: Management of Microbiological Hazards in Foods Wageningen, The Netherlands, March 2004	1
VLAG course: Food Fermentation Wageningen, The Netherlands, October 2004	1
VLAG course: Regulation of Food Intake and its Implications for Nutrition and Obesity Wageningen, The Netherlands, April 2006	1
Meetings of learned societies:	
The Fifteenth International Plant Nutrition Colloquium, Beijing, China, September 2005	1
Zinc Crops 2007 Conference, Istanbul, Turkey, May 2007	1
Training courses:	
Review of literature and preparation of proposal Wageningen, The Netherlands, October, 2002 – January, 2003	5
General courses:	
Presentation techniques:	
Presentations at the Group of Food Microbiology and INREF program, Wageningen, The Netherlands, 2002-2007	1
Didactical:	
Supervision of BSc students (20) at the College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China, 2003-2007	2
Supervision of MSc students (3) at the College of Food Science and Nutritional Engineering, China Agricultural University, Beijing, China, 2005-2007	2
Techniques for writing and presenting scientific papers	
Course for PhD students given by graduate school of CAU: English writing skills China Agricultural University, Beijing, China, September 2005–January 2006	2
PhD student weeks:	
PhD student week organized by INREF, Wageningen, The Netherlands, November, 2002	1
Workshop organized by CAAS and WUR, Beijing, China November, 2003	1

PhD study trip organized by the WU Laboratory of Food Microbiology, South Africa, March 2005 2

Optional:

Teaching assistant:

Course of Bioprocessing and Food Quality, Beijing, China, May 2006 and 2007 2

Teaching:

BSc Course: Technology of Baked Food, Beijing, China, 2003-2007 once yearly 5

Msc Course: Advances in the Chemistry of Natural & Bioactive Products, 2005- 07 once yearly 1

Undergraduate course:

Food Fermentation Wageningen, The Netherlands September 2004 2

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“From Natural Resources to Healthy People”

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The micronutrient malnutrition problem

Chronic micronutrient deficiencies, particularly of vitamin A, iron and zinc, lead to impaired mental and physical development and decreased work output, and contribute to morbidity from infections. Pregnant women and children are vulnerable groups. Animal products are good sources of desired micronutrients, but most people in West Africa and China depend largely on sorghum and rice, respectively, for their daily food. These plant-based foods contain limited amounts of micronutrients while they also contain anti-nutritional factors such as phytic acid and polyphenols that inhibit absorption of micronutrients by humans.

Next to the nutritional quality, the production of enough food is an important problem as population growth leads to higher demands for food and more permanent cropping, both increasing pressure on natural resources. In West Africa, soil and water conservation measures are being developed to prevent soil erosion, nutrient and water losses and to maintain or even increase yields. In China, the introduction of aerobic rice systems aim to reduce water use per kg of rice, maintaining yields similar to the current flooded rice systems.

Programme strategies to improve the supply of micronutrients

The increasing demand for food stipulates that improvements in food quality cannot be accepted when they are at the expense of food quantity. Any solution should be in line with sustainable natural resource management.

The programme applied a food chain approach (figure) in sorghum and (aerobic) rice to explore synergies and trade-offs between different interventions along the chain.

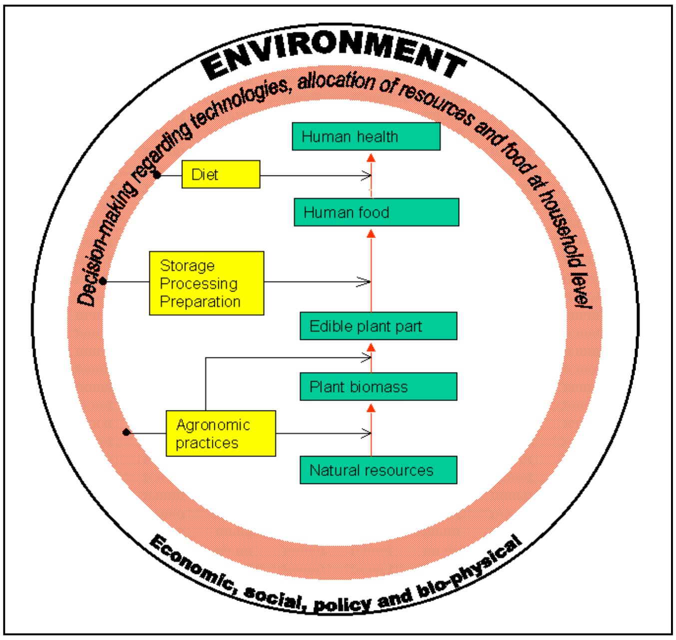

Diagram of the food chain

The food chain approach is indicated showing how external conditions like the economic and bio-physical environment set the stage for decision making at the household level. These decisions in their turn determine practices which have a direct impact on the processes at different points in the food chain. Research in the programme has been done related to each of the three types of interventions.

Agronomic practices should aim to increase uptake and allocation of micronutrients from soil to edible plant parts, while keeping accumulation of anti-nutritional factors low. Research has focussed on effects of genotype, environment & management and their interaction on micronutrient/phytic acid molar ratio in seed. This has led to recommendations on choice of genotype, fertiliser and water use.

Food processing aims to concentrate desired micronutrients in end products and inactivate anti-nutritional factors. Research focussed on effects of milling and processing on micronutrient/phytic acid molar ratio in food, leading to recommendations on optimal combinations of unit operations.

Nutrition studies aim to validate the results in humans. Research focussed on dietary composition, determination of methods to measure impact and evaluation of effects of some of the proposed changes upstream in the food chain on micronutrient uptake in vulnerable groups. This has led to insight in sources of micronutrient and anti-nutritional factors and in the potential contribution of an intervention in the staple food.

At the end of the programme an analysis will be made to determine the relative impact of the different proposed measures along the chain for the final aim: improved micronutrient nutrition of targeted vulnerable groups.