Original Article

Household dietary strategies to enhance the content and bioavailability of iron, zinc and calcium of selected rice- and maize-based Philippine complementary foods

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Abstract

Philippine complementary foods are predominantly plant-based, with a low content of readily available iron, zinc, and calcium, and a relatively high amount of phytate, a potent inhibitor of mineral absorption. Some of the phytate is water soluble, and hence can be removed by soaking. In this study we have compared the iron, zinc, and calcium content, and estimated iron and zinc bioavailability of rice- and maize-based Filipino complementary foods prepared with and without soaking and/or enrichment with chicken liver, egg yolk, small soft-boned fish, and mung bean grits. Analysis of iron, zinc, and calcium were performed by atomic absorption spectrometry, and phytate (based on hexa-(IP6) and penta-inositol phosphate (IP5) by HPLC; corresponding [Phy]/[Fe] and [Phy]/[Zn] molar ratios were calculated as predictors of iron and zinc bioavailability. Addition of chicken liver, followed by egg yolk, resulted in the greatest increases in iron and zinc content for both the rice- and maize-based complementary foods, whereas addition of small dried fish with bones had the greatest effect on calcium. The IP5 + IP6 content and [Phy]/[Zn] molar ratios were higher in the maize- than rice-based complementary foods, and were reduced by soaking, although only the maize plus mung bean grits, with and without soaking, had [Phy]/[Zn] molar ratios above 15. Enrichment with animal protein or soaking has the potential to enhance the content of absorbable iron, zinc, and probably calcium to varying degrees in rice- and maize-based Philippine complementary foods.

Keywords: dietary strategies, Philippine complementary foods, iron, zinc, calcium.

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Introduction

The nutritional status of Filipino infants and pre-school children is poor as shown by Philippine national nutrition surveys. The prevalence of anemia and vitamin A deficiency is high among children less than 5 years of age (i.e. 30%) (Kuizon *et al*., 1997; Cheong *et al*., 2001; Madriaga *et al*., 2001) and approximately 32% are underweight or stunted; only

7% are wasted (Laña *et al*., 2001). Such a high prevalence of malnutrition may be explained, in part by inadequacies in dietary intakes. The diets of children 0–3 years are known to be predominantly plant based (Villavieja *et al*., 1998). Hence, they have low levels of readily available zinc and haem iron from flesh foods, and relatively high amounts of phytates and polyphenols (Lasztity & Lasztity, 1990), known to inhibit the absorption of iron, zinc, and to a lesser extent calcium in adults, by forming insoluble complexes in the gastrointestinal tract (Lönnerdal *et al*., 1989; Heaney *et al*., 1991; Brune *et al*., 1992; Hurrell *et al*., 2003).

Recent research, however, has confirmed that soaking can be used to reduce the phytate content of maize, rice, and certain legume flours mainly by passive diffusion of water soluble sodium, potassium, or magnesium phytates (de Boland *et al*., 1975; Chang *et al*., 1977). Soaking rice and mung bean flour for 6 h, can reduce phytate content by as much as 65% and 47% for the rice and mung bean flour, respectively (Perlas & Gibson, 2002). Soaking of maize flour for one hour reduces phytate content by 12% if the soaking water is removed by decanting (Hotz *et al*., 2001; Temple *et al*., 2002). Soaking is both practical and acceptable and can be readily undertaken in the home (Hotz & Gibson, 2001).

The Food and Nutrition Research Institute (FNRI) of the Philippines has recommended some easy to prepare foods for infant and young child feeding based on dishes that are cooked for family use (FNRI, 1998). These dishes are presumed to have a high energy and nutrient density and aim to improve the nutrient adequacy of the complementary diets of infants and young children. Some of the strategies suggested by FNRI include enrichment of the rice-based complementary foods with mung beans, or animal source foods such as egg yolk and fish. Addition of small fish, which can be eaten whole with bones, is especially useful because they increase the available calcium and zinc as well as phosphorus content of complementary foods (Hansen *et al*., 1998; Martinez *et al*., 1998; Larsen *et al*., 2000). Addition of chicken liver to complementary foods has also been recommended by WHO (1998, 2000) in an effort to meet the iron and zinc requirements of infants and young children. This is important because iron and zinc as well as calcium have all been identified as 'problem nutrients' by WHO (1998) in terms of meeting their estimated needs from complementary foods. Chicken liver not only contains large amounts of bioavailable haem iron and to a lesser extent zinc, but like fish protein, it may also improve the absorption of both non-haem iron and zinc in a meal to some extent (Cook & Monsen, 1976; Sandström & Cederblad, 1980). Further, chicken liver is readily available, affordable, and consumed by some young children in the Philippines.

Therefore, the overall objective of this study was to assess the impact of dietary strategies designed to enhance the content and bioavailability of iron, zinc, and calcium in complementary foods used for infant and child feeding in the Philippines. Specifically, we have analysed and compared the iron, zinc, and calcium, and penta-(IP5) and hexa-inositol (IP6) phosphate content, and molar ratios of [phytate]/[iron] and [phytate]/[zinc] of selected rice- and maize-based complementary foods, with and without soaking and/or enrichment with animal source foods.

Materials and methods

Materials

Polished white rice (Oryza sativa L), fresh chicken liver, chicken eggs, and mung beans (Phaseolus aureus) (yellow variety) were bought from the local supermarket in Dunedin, New Zealand. The rice was a product of Thailand. Dried fish (Anchovy long jawed) was bought from an Asian Store in Dunedin. White maize grits (Zea mays L) were obtained from the Philippines. Maize grits were prepared by milling white maize and sieving it through a size 12 sieve. The same batch of rice, maize, mung beans, and other enrichment ingredients were used in the preparation and analysis of both the un-soaked and soaked complementary foods.

All the laboratory analysis were undertaken in the Trace Element Laboratory of the Department of Human Nutrition, University of Otago, New Zealand.

Preparation of complementary foods

The proportions of the ingredients for all the recipes – soft cooked rice; fish; egg yolk; mung beans – are based on those used for complementary foods for 4–12 month old breast-fed Filipino infants (FNRI, 1998) and compiled by the FNRI of the Philippines. The quantity of chicken liver used was based on the amount required to meet the estimated needs for zinc from complementary foods of infants 9–11 months of age (WHO, 1998). These same proportions of fish, egg yolk, and mung beans were also used for the preparation of the maize-based complementary foods, also used for infant and child feeding in certain areas of the Philippines.

The effect of soaking was tested in all the complementary foods based on maize, but only in the rice-based complementary food enriched with mung beans. Before preparing the porridges, both the polished rice and maize grits were thoroughly washed twice with distilled, deionized water as practiced in a Filipino household. Soaked ingredients were prepared by incubating 1 part maize or mung bean grits to 4 parts distilled water, at 30°C water bath for 6 h.

Preparation of cooked complementary foods for laboratory assay

The cooked complementary foods were freeze dried (Free Zone 6 L, Freeze Dry System Model 77530, Labconco, Kansas City, MO, USA) in preparation for analysis of total iron, zinc, and calcium, and analysis of IP5 and IP6. The freeze-dried complementary foods were then milled in an agate ball mill (Model 2 MN @ Brinkmann Instruments Division, Sybron, Canada) to obtain a fine powder. All glassware used for the analytical methods was soaked in acid (10% HNO_3) and rinsed with distilled deionized water to avoid adventitious contamination.

Determination of moisture

Fifty gram aliquots from the cooked porridges were freeze dried to constant weight for the determination of dry matter content. These freeze-dried aliquots were also used for the mineral and IP5 and IP6 assays.

Determination of total iron, zinc, and calcium content of cooked complementary foods

This procedure involved dry ashing the freeze-dried aliquots of the cooked complementary foods, followed by the extraction of the minerals and their subsequent analyses by flame atomic absorption spectrometry (AAS) (Perkin 1982). Five-gram samples of the freeze-dried cooked porridges were used for the mineral analysis (AOAC, 1993). Iron, zinc, and calcium were directly assayed on aliquots of the ashed solution, as described earlier (Ferguson *et al*., 1993). Concentrations of iron, zinc, and calcium in the samples were calculated from the regression equations derived from the concentrations of standard solutions (BDH Laboratory supplies) and their corresponding absorbance.

Accuracy and precision of all the AAS methods were checked by analysing replicates of two standard reference materials (SRM #1568a and SRM #1572). Mean analysed values were within the certified values for iron, zinc, and calcium in the standard reference materials. Mean (±SD) and coefficient of variation (CV as percentage) for analysed values were: iron ($n = 54$) 6.9 ± 0.60 μg/g; CV = 8.8%, zinc ($n = 43$) 20.0 ± 0.57 μg/g; CV = 2.8%, and percentage recovery for calcium ($n = 44$) 99 ± 8.3%; CV = 8.4%. Certified SRM values were: iron 7.4 ± 0.9 μg/g, zinc 19.4 ± 0.5 μg/g, and percentage recovery for calcium 100%.

Determination of penta- and hexa-inositol phosphate

The High Performance Liquid Chromatography (HPLC) procedure of Lerhfeld (1989) was followed for the analysis of IP5 and IP6, as modified by Hotz and Gibson (2001). Approximately 0.5 g of each freeze-dried sample was analysed for the inositol phosphates using a Waters 2690 Separations Module (Waters, Milford, MA, USA), separated by a Hypersil column (H30DS-250A, HiCHROM, Berkshire RG7 4PE, England) and detected with a differential refractometer (410 Differential Refractometer, Waters, MA, USA). Concentrations of the IP5 and IP6 were calculated from regression equations derived from the different concentrations of the standard solutions and peak areas of the samples.

Accuracy and precision of this HPLC procedure was assessed through interlaboratory analysis of the IP5 + IP6 content of wheat bran and an unrefined white maize. The maize flour analysed in our laboratory gave a mean value of 586 ± 63 mg/100 g IP5 + IP6 ($n = 14$) compared to the reported value of 660 mg/100 g analysed in another laboratory. The wheat bran analysed in our laboratory had an IP5 + IP6 ($n = 14$) content of 3295 ± 102 mg/100 g compared to the reported value of 3348 mg/100 g.

Results

Iron, zinc, and calcium content of selected rice- and maize-based Philippine complementary foods

The rice- and maize-based complementary foods included here are those recommended for feeding nine-month old Filipino infants (FNRI, 1998); their total iron, zinc, and calcium contents are shown in Table 1. The iron, zinc, and calcium content of the un-soaked and soaked complementary foods were not significantly different. Hence, in Table 1 their mean (±SD) iron, zinc, and calcium are presented. The dry matter content of these foods ranged from 18 to 26%.

The addition of chicken liver resulted in the greatest increase in the iron and zinc content of the rice-based complementary foods (Table 1), followed by that of egg yolk, whereas the addition of small fish including bones increased their calcium content the most. Addition of soaked compared with un-soaked mung bean grits had no effect on the iron, zinc, and calcium content of the rice-mung bean mixture. Results for the un-soaked and soaked maize-based complementary foods showed the same trend (Table 1), with the foods with added chicken liver again having the highest iron and zinc content and those with added fish having the highest calcium content.

Penta- and hexa-inositol phosphate, [Phy]/[Fe] and [Phy]/[Zn] molar ratios of selected rice- and maize-based complementary foods

Penta-and hexa-inositol phosphate content of all the rice- and maize-based complementary foods included in this study were also analysed; total IP5 + IP6 and their corresponding [Phy]/[Fe] and [Phy]/[Zn] molar ratios are presented in Table 2. The [Phy]/[Zn] molar ratio provides an indirect estimate of the proportion of absorbable zinc, whereas the [Phy]/[Fe] molar ratio has been used as a predictor of iron bioavailability.

Rice plus un-soaked mung bean grits had the highest IP5 + IP6 content (i.e. 275.6 ± 12.5 mg/100 g) that was reduced significantly (151.0 ± 12.5 mg/100 g, $P < 0.001$) by 45% after soaking mung beans for six hours (Table 2). By contrast, rice porridge un-enriched or enriched with liver, fish, and egg yolk had a very low IP5 + IP6 content. Note that the maize-based complementary foods had a higher content of

Table 1. Mean (SD) of iron, zinc and calcium content of selected rice-and maize-based complementary foods (mg/100 g dry wt)

Composition	Iron	Zinc	Calcium
Rice-based			
Rice	0.45 ± 0.01 (3)	1.53 ± 0.01 (3)	5 ± 1 (3)
Rice + Liver	10.20 ± 0.90 (3)	5.30 ± 0.36 (3)	11 ± 1 (3)
Rice + Fish	2.15 ± 0.12 (3)	3.50 ± 0.23 (2)	663 ± 2 (3)
Rice + Egg yolk	4.93 ± 0.13 (3)	4.52 ± 0.9 (3)	138 ± 10 (3)
Rice + Mung Bean	1.53 ± 0.20 (5)	1.93 ± 0.07 (6)	29 ± 1 (6)
Maize-based			
Maize	0.81 ± 0.24 (6)	0.42 ± 0.07 (6)	3 ± 0.6 (6)
Maize + Liver	9.36 ± 1.34 (6)	4.25 ± 0.90 (6)	13 ± 1.1 (6)
Maize + Fish	2.74 ± 0.62 (6)	2.53 ± 0.16 (6)	839 ± 61 (6)
Maize + Egg yolk	5.01 ± 0.64 (6)	3.39 ± 0.29 (6)	120 ± 4 (6)
Maize + Mung beans	2.19 ± 0.06 (6)	1.14 ± 0.19 (6)	33 ± 0.3 (6)

( ) number of samples analysed.

Table 2. Mean (SD) of inositol penta- and hexa phosphate content, [Phy]/[Fe] and [Phy]/[Zn] molar ratios of selected rice and maize-based complementary foods†

Composition	IP5 + IP6 (mg/100 g)		[Phy]/[Fe]		[Phy]/[Zn]	
	Un-soaked	Soaked	Un-soaked	Soaked	Un-soaked	Soaked
Rice-based						
Rice	18.8 ± 5.4 (4)		3.5 ± 1.0		1.2 ± 0.4	
Rice + Liver	4.3 ± 1.1 (4)		0.1 ± 0.0		0.1 ± 0.0	
Rice + Fish	10.0 ± 5.7 (4)		0.4 ± 0.2		0.3 ± 0.2	
Rice + Egg Yolk	9.9 ± 4.3 (3)		0.2 ± 0.0		0.2 ± 0.1	
Rice + Mung Beans	275.6† ± 12.5 (4)	151.0 ± 12.5 (4)	16.7† ± 0.8	7.4 ± 0.3	13.8† ± 0.6	8.1 ± 0.4
Maize-based						
Maize-based	62.1* ± 8.7 (3)	31.6 ± 2.4 (3)	8.0 ± 2.9	4.7 ± 1.0	12.7 ± 1.8	10.5 ± 2.2
Maize + Liver	25.7 ± 0.3 (2)	21.1 ± 1.1 (2)	0.2 ± 0.0	0.2 ± 0.0	0.7* ± 0.0	0.4 ± 0.0
Maize + Fish	40.9* ± 2.6 (2)	23.6 ± 2.1 (2)	1.4* ± 0.1	0.7 ± 0.1	1.5* ± 0.1	0.9 ± 0.1
Maize + Egg Yolk	28.7 ± 2.0 (2)	21.7 ± 1.7 (2)	0.4 ± 0.1	0.4 ± 0.0	0.9 ± 0.1	0.6 ± 0.1
Maize + Mung Bean	367.5 ± 17.0 (2)	352.6 ± 8.6 (2)	14.0 ± 0.6	13.8 ± 0.3	37.9* ± 1.8	26.5 ± 0.6

*Un-soaked values significantly higher than soaked values at $P < 0.05$; †Un-soaked values significantly higher than soaked values at $P < 0.001$. ( ) number of samples analysed.

IP5 + IP6 than the rice-based complementary foods. Again, as was noted for the rice plus mung bean grits, the combination of maize plus un-soaked mung bean grits had the highest IP5 + IP6 (Table 2). Soaking of both the maize and mung bean grits, resulted in a slight non-significant decrease in phytate content, that is, 367.5 ± 17.0 mg/100 g and 352.6 ± 8.6 mg/100 g for the un-soaked and soaked maize + mung bean, respectively. In general, the IP5 and IP6 content of all the maize porridge combination decreased after soaking for six hours (Table 2).

The inhibitory effect of phytate on non-haem iron is known to be dose dependent (Hallberg *et al*., 1989; Hallberg & Hulthén, 2000). However, the critical [Phy]/[Fe] molar ratio above which bioavailability of non-haem iron in a meal is compromised has not been well established yet, but Hurrell (2003) has suggested that iron absorption is increased only if the phytate to iron molar ratio is reduced to <1 or preferably <0.4. Nevertheless, bioavailability of iron is likely to be more compromised in the rice : mung bean grits combinations prepared with un-soaked mung bean grits with a high phytate content than the mixture prepared with soaked mung bean grits, followed by rice alone (Table 2). Note the very low [Phy]/[Fe] molar ratio for rice enriched with fish, liver, or egg yolk. With the exception of the maize and un-soaked mung bean grits mixture, all the other maize-based complementary foods (Table 2) had higher [Phy]/[Fe] molar ratios than the corresponding rice-based complementary foods (Table 2). Soaking resulted in a lower [Phy]/[Fe] molar ratio for both the maize porridge and the maize plus fish combination, but had no impact on the [Phy]/[Fe] molar ratios of the other enriched maize-based porridges.

None of the rice-based complementary foods had a [Phy]/[Zn] molar ratio > 15, the critical value above which the absorption of zinc is reportedly severely compromised (Turnland *et al*., 1984) (Table 2), although the molar ratio of the rice plus un-soaked mung bean grits mixture was approximately 14. Among the maize-based complementary foods, the maize plus both the soaked and un-soaked mung bean grits combination had [Phy]/[Zn] molar ratios above 15 (i.e. 38 and 26, respectively). Soaking resulted in lower molar ratios for the maize-based complementary foods, although for the soaked maize and mung bean grits mixture, the [Phy]/[Zn] molar ratio was still above the critical value of 15.

Discussion

The World Health Organization (1998) has defined iron, zinc, and calcium as 'problem nutrients' in many complementary diets in developing countries, because of the large discrepancy between the amount of these inorganic nutrients in complementary foods compared to the corresponding estimated needs of the infant from complementary foods (WHO, 1998; Dewey & Brown, 2003). A similar trend has been identified for the densities of iron, zinc, and calcium in complementary foods compared to their corresponding desired nutrient densities (WHO, 1998; Dewey & Brown, 2003). Consequently, in this study we have analysed the iron, zinc, and calcium content of complementary foods recommended for infant and child feeding in the Philippines together with their content of phytic acid, known to have a potent inhibitory effect on the bioavailability of these two trace elements, as well as calcium.

The FNRI has recommended modifications to family meals to improve nutrient intakes of Filipino breast-fed infants receiving complementary foods (FNRI, 1998). Recommendations include the addition to soft cooked rice, of an egg yolk for breakfast, flaked fish for lunch, and mashed mung beans for dinner. Helen Keller International (HKI), Philippines has also launched the 'Weaning Moments Program' which also aimed at improving complementary feeding practices via nutrition communication (Klemm *et al.*, 1977). The main message of the programme was the adoption of the '1 + 3 Complementary Mix'; 1 being *lugao* (rice porridge) as the main food, with 3 representing a combination of a high energy food such as oil, high protein food such as fish, and a vitamin-rich food such as mashed vegetables or fruits. The World Health Organization (1998) has compiled a list of candidate foods known to be a rich source of iron, zinc, and calcium. They include chicken liver as a rich source of iron, and fish as a moderate source of iron and zinc, and fish when eaten whole with bones (such as sardines or dried fish) as a good source of calcium.

Content and relative availability of iron in selected rice- and maize-based complementary foods

Meat and poultry and to a lesser extent fish, contain relatively high amounts of readily absorbable haem iron and zinc. Further, addition of even small amounts of cellular animal protein can enhance the absorption of non-haem iron and zinc, possibly by the formation of soluble zinc and iron complexes with L-amino acids such as cysteine as well as peptides (Taylor *et al.*, 1986; Sandström *et al.*, 1989).

Our laboratory results are consistent with the updated recommendation by WHO (1998) that the addition of chicken liver to a complementary diet based on rice or maize would markedly enhance a child's iron intake (Table 1). More modest increases in iron intake can be achieved by enriching rice or maize-based porridges with egg yolk, and to a lesser extent fish. Note the much smaller increases in iron content when maize and rice-based complementary foods are enriched with mung bean grits. It is well established, however, that the adequacy of dietary iron in complementary foods depends not only on their total iron content, but also on their bioavailability. The latter is affected by certain dietary components known to inhibit and enhance non-haem iron absorption (Hallberg & Hulthén, 2000). Of the inhibitors, phytates found in rice and unrefined maize (Hallberg *et al.*, 1989; Sandberg *et al.*, 1989), the two major staples of complementary diets consumed in the Philippines, have a major inhibiting effect on non-haem iron absorption in both adults (Hallberg *et al.*, 1989; Sandberg *et al.*, 1999; Hurrell *et al.*, 2002, 2003) and infants (Davidsson *et al.*, 1996). Further, their inhibitory effect is dose-dependent (Hallberg & Hulthén, 2000). Nevertheless, to date, the phytate content of complementary foods used in the Philippines has not been characterized. In this study, we have analysed two of the higher inositol phosphates: inositol penta- and hexa-phosphate, known to be the strongest antagonists of non-haem iron absorption (Brune *et al.*, 1992; Sandberg *et al.*, 1999). Note that all the maize-based complementary foods analysed in this study had a higher IP5 + IP6 content than those based on rice (Table 2), a finding consistent with literature data (Gibson *et al.*, 1998).

In this study, the phytate content of the un-soaked rice and maize-based complementary foods was relatively low compared to those values from other parts of the world (Lasztity & Lasztity, 1990; Hotz *et al.*, 2001). In the Philippines, milling and sieving is used to process maize grits, and both polished rice and maize grits are commonly washed before cooking, all practices known to reduce the phytate content of maize (Lasztity & Lasztity, 1990; Hotz *et al*., 2001) and rice (Perlas & Gibson, 2002).

Soaking mung bean grits resulted in an almost 50% reduction in the phytate content of the rice-based complementary food prepared with un-soaked mung bean grits (Table 2), but only a 4% reduction in that prepared with maize. However, soaking maize grits for six hours followed by decanting the soaking water prior to preparing the porridges led to consistent reductions in the IP5 + IP6 content of each of the maize-based complementary foods. Nevertheless, the magnitude of the reductions achieved varied, ranging from 42% for the soaked maize + fish to only 18% for maize + chicken liver. The mechanism whereby the phytate content is reduced after soaking maize in water, followed by removal of the soaking water is attributed mainly to diffusion of water soluble sodium, potassium, or magnesium phytate. This occurs because up to 70% of the phytate present in maize (de Boland *et al*., 1975), and a large part of the phytates in rice and certain legumes, is water soluble (Cheryan, 1980). Hydrolysis of phytic acid (inositol hexa-phosphate) via the activity of endogenous phytatse enzyme (myo inositol hexaphosphate phosphorylase EC 3.1.3.26) is unlikely because maize and rice are both low in phytases (Egli *et al*., 2002).

Reduction in the IP5 and IP6 content of maize porridges via soaking alone, or combined with enrichment with iron-rich ingredients such as chicken liver, and to a lesser extent egg yolk and fish, has resulted in a dramatic reduction in their corresponding [Phy]/[Fe] molar ratios (Table 2). As yet there is no consensus on the critical cut-off for [Phy]/[Fe] molar ratios, above which iron absorption is reportedly impaired. Indeed, because the inhibitory effect of phytate on non-haem iron absorption is known to be dose dependent (Hallberg *et al*., 1989; Sandberg *et al*., 1999), a critical cut-off point may be difficult to define.

However, Hurrell *et al*. (2002) have suggested that phytic acid begins to lose its inhibiting effect on non-haem iron absorption when [Phy]/[Fe] molar ratios are less than 1.0 : 1.0, although to achieve the maximum benefit (i.e. five fold increase in iron absorption), it appears that all the phytic acid should be removed or degraded (Hurrell, 2003). Because the latter is difficult to achieve, Hurrell (2003) recommends reducing [Phy]/[Fe] molar ratios in complementary foods to below 1.0 : 1.0, and preferably below 0.4 to achieve about a twofold increase in iron absorption. Note that most of the [Phy]/[Fe] molar ratios presented here for the un-soaked rice-based porridges are less than 1.0. Exceptions are those enriched with mung beans and un-enriched [Phy]/[Fe] molar ratios > 1.0), and rice porridge enriched with fish [Phy]/[Fe] molar ratio = 0.4). In contrast, for the un-soaked maize-based porridges only the one enriched with egg yolk had a [Phy]/[Fe] molar ratio < 1.0 but = 0.4, whereas that enriched with liver had a [Phy]/[Fe] molar ratio < 0.4. Moreover, soaking the maize-based porridges enriched with liver and egg yolk did not result in any further reduction in their [Phy]/[Fe molar ratios, although when they were enriched with fish, the [Phy]/[Fe] molar ratio was less than 1.0 after soaking. Some caution is needed when interpreting these [Phy]/[Fe] molar ratios, especially for the porridges enriched with chicken liver as the bioavailability of ferritin iron in chicken liver may be poor.

Polyphenols and tannins are also known to inhibit non-haem iron absorption, forming insoluble iron-phenolic compounds (Hurrell *et al*., 2003). They are found in certain cereals, notably red sorghum and bull rush millet, but not in rice or white maize, and hence were not analysed in the study reported here. Egg yolk is also known to contain a component, phosvitin, that inhibits the absorption of non-haem iron in adults (Cook & Monsen, 1976). Whether comparable results would be observed with infants is still uncertain. To our knowledge, there are no reports among infants on the bioavailability of iron from complementary foods based on egg yolks. In some studies, the efficacy of consumption of egg yolks on iron status during infancy has been investigated but results have been mixed (Guldan *et al*., 2000; Makrides *et al*., 2002). Nevertheless, egg yolk does provide an important source of several other micronutrients besides iron, often limiting in complementary diets, including riboflavin, vitamin B-12, preformed vitamin A, calcium, and zinc.

Of the dietary components known to enhance non-haem iron absorption (i.e. cellular animal protein, ascorbic acid and other organic acids: citric, lactic, malic and tartaric), cellular animal protein is probably the only factor of significance in the recipes developed by FNRI for infant and child feeding. Absorption of non-haem iron is always lower than that of haem iron, ranging from 2% to 20% depending on a person's iron status, and the content of dietary enhancers and inhibitors in a meal. By contrast, absorption of haem iron, found exclusively in cellular animal protein, is much higher than non-haem iron, ranging from 15 to 25% in normal persons and 25% in iron-deficient persons. It is thus evident that many other dietary components besides phytate influence the absorption of iron, so that the effect of phytate alone on iron absorption is difficult to establish. As a result, the [Phy]/[Fe] molar ratios are probably a less valid predictor of iron bioavailability than corresponding ratios for zinc.

Content and relative availability of zinc in selected rice- and maize-based complementary foods

Our results confirm that enriching both rice- and maize-based porridges with chicken liver, and to a lesser extent egg yolk, markedly enhances their zinc content to almost three times (Table 1). Enrichment with fish also enhances the total zinc content of these two porridges but to a lesser extent, whereas the addition of mung bean grits had a much more modest effect, especially for the porridge based on rice. Further, because mung beans have such a high IP5 + IP6 content, the [Phy]/[Zn] molar ratio of maize porridge enriched with mung beans is dramatically increased to a level above which zinc bioavailability is said to be compromised (Table 2). Soaking mung bean grits resulted in some reduction in the corresponding [Phy]/[Zn] molar ratios, although values for the maize-based porridges were still above the critical value (>15). By contrast, none of the other enriched porridges, irrespective of whether they were based on maize or rice, had [Phy]/[Zn] molar ratios said to inhibit zinc absorption.

The adverse effect of phytate on zinc absorption has been confirmed by several *in vivo* studies in adults (Turnland *et al*., 1984; Adams *et al*., 2002), and more recently in one study in infants (Davidsson *et al*., 2004). Moreover, in studies of adults consuming day-long test meals of tortillas made from either low-phytate maize hybrids or wild-type unmodified maize (Hambidge *et al*., 2004), the magnitude of the adverse effect of phytate on zinc absorption was dependent on the extent of the phytate reduction. Nevertheless, whether such an adverse effect also occurs in weanlings in developing countries remains uncertain (Manary *et al*., 2000). Clearly, addition of mung bean grits to rice or maize-based porridges for complementary feeding is not advisable if suboptimal zinc status is a concern.

Content and relative availability of calcium in selected rice- and maize-based complementary foods

Small fish, when eaten whole with the bones, can provide a rich source of calcium (WHO, 1998, 2000). Our analytical results have confirmed these findings. Both maize and rice porridges enriched with whole, powdered anchovies, had very high calcium content (663 and 784 mg/100 g, respectively) (Table 1). Moreover, calcium from small whole fish with bones appears to be readily absorbed. Hansen *et al*. (1998) showed that calcium retention from soft boned fish was comparable to that from skimmed milk (i.e. $23.8 \pm 5.6\%$ versus $21.8 \pm 6.1\%$), based on measurement of whole body retention of calcium in an *in-vivo* human adult study. Similar findings were reported by Larsen *et al*. (2000).

Enrichment of the rice- and maize-based porridges with egg yolk also increased their calcium content by 28 and 31 times, respectively, whereas only modest increases in calcium were achieved when mung beans, and to a lesser extent, chicken liver were used (Table 1). Data on the bioavailability of calcium from specific foods are limited, and published results are inconsistent, making it difficult to establish the bioavailability of calcium from these foods (COMA 1991). It is likely, however, that the bioavailability of calcium from the maize porridge with and without

Table 3. Calculated iron, zinc, and calcium intakes (mg/d) from rice-based complementary foods for a 10-month old Filipino infant compared with the WHO* estimated needs (as percentage)

			Iron		Zinc		Calcium	
Breakfast	Lunch	Dinner	mg	% WHO estimate	mg	% WHO estimate	mg	% WHO estimate
+Egg	+Fish	+Liver	12.7	116	9.67	371	563	125
+Egg	+Fish	+Mung Beans	6.1	56	6.74	259	570	127
+Egg	+Liver	+Mung Beans	12.1	111	8.28	318	126	28

*Dewey and Brown (2003).

added mung bean grits is poor, based on balance study data by Rosado *et al*. (1992), attributed in part to their high phytate content. The latter is known to inhibit the absorption of calcium (Heaney *et al*., 1991). Hence, the reduction in IP5 + IP6 levels achieved by soaking maize and mung bean grits (Table 2) would likely enhance calcium absorption to some degree.

Some caution should be used when interpreting the results presented here because many of the *in vivo* bioavailability studies cited were conducted on adults rather than on infants. Nevertheless, it appears that by using a combination of these enriched complementary foods, the estimated needs for iron, zinc, and calcium set by WHO for a 10 month-old Filipino infant can be met (Dewey & Brown, 2003). Table 3 presents intake data for an ideal one-day menu for an infant aged 10 months with an average consumption of breastmilk, and confirm that selection of rice and egg yolk for breakfast, fish for lunch, and chicken liver for dinner is the optimal combination. Intakes of iron, zinc, and calcium provided by this menu are all well above the WHO estimated needs (Dewey & Brown, 2002), provided moderate bioavailability is assumed for intakes of iron and zinc. Note that the estimated needs for zinc appear to be met by all the menus presented in Table 3.

Conclusion

Enrichment with animal source foods such as chicken liver, small dried fish with bones, and egg yolk can increase the content of iron, zinc, and calcium to varying degrees in selected rice- and maize-based complementary foods used for infant and child feeding in the Philippines. Soaking can reduce the phytate content of maize-based complementary foods, thus having the potential to enhance the bioavailability of iron, zinc, and probably calcium in these complementary foods. Nevertheless, the bioavailability of these inorganic nutrients in complementary foods enriched with mung beans is still likely to be low, but could be increased for iron by incorporating pureed ascorbic acid-rich fruits or vegetables with the maize-mung bean porridge. Soaking is a simple strategy that can be practised at the household level. Thus, promotion of enrichment and soaking strategies may be an affordable way of reducing the high prevalence of anemia and stunting in infants and young children in the Philippines. Biochemical and functional indices of calcium deficiency in Filipino children have not been assessed but inadequacies of calcium are likely in diets of infants and young children receiving complementary foods in the Philippines. Therefore, increasing the calcium content of complementary foods by the use of powdered small fish with bones would ensure that infants and young children meet their estimated calcium needs from complementary foods. These enrichment strategies are practical and affordable in many South-east Asian countries, and their adoption would also simultaneously enhance the intakes of several other micronutrients, often limiting in complementary diets (e.g. riboflavin, niacin, vitamin B12, and preformed vitamin A).

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