Improving micronutrient status of the Sri Lankan population: effect of iron and zinc fortified rice flour

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Abstract
In Asia, rice provides more than 70% of the energy in the diet. Rice is the single most important crop occupying 34.0% of the total cultivated area in Sri Lanka. About 1.8 million farm families are engaged in paddy cultivation island-wide, produce 2.7 million tonnes of paddy annually and satisfy around 95.0% of the domestic requirement. As such, rice can be a good candidate for fortification with micronutrients because it is the staple diet of all sectors of the population, consumed almost daily in large amounts in Sri Lanka. This intervention programme describes the feasibility of rice flour fortification to increase the micronutrient intake and thereby to improve growth and status of iron, zinc and folate of Sri Lankan population.

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This study was performed in University of Ruhuna, Sri Lanka and the results were included in a thesis with eight published papers. Further, two orations were done at the University Academic Sessions & Sri Lanka Medical Association respectively and recognized as best post graduate research by the Hiran Thilakerathne fund of the University Grants Commission, Sri Lanka Association for Advancement of Science (SLAAS) and Thrid World Academy of Sciences (TWAS) & National Science Foundation of Sri Lanka during respective years and defended the thesis on 01 of February 2007

Introduction

Food fortification is an important aspect of a nutritional intervention programme. The fortified food products are expected to become the main source of the specific added nutrient in the targeted population, and help prevent inadequacies of those nutrients in groups at risk. The effectiveness of such a programme depends on whether or not the fortified food is accepted, purchased, and consumed by them. Further factors such as the quality, taste, and price of the fortified products also will play important roles. Fortification programmes make a range of effective investments that improve intake and status of micronutrients, contribute to size and stature of the population (direct effects), and improve cognitive ability as well as scholastic achievements (indirect effects). Further, economic growth and poverty reduction can also be achieved through iron and zinc deficiency reduction (1).

As in the other countries in Asia, Sri Lanka faces widespread health and nutritional problems. Micronutrient deficiencies strike both children and women and surveys showed that deficiencies in iron, iodine, and vitamin A are the most damaging forms (2). Anaemia is a major public health problem in Sri Lanka, affecting all segments of the population and contributing to increased morbidity and mortality rates. In 2001 the prevalence was estimated as 32% among non-pregnant women, 30% among pregnant women, 22% among adolescents, 21% among primary schoolchildren, and 30% among preschool children (3). There have been no reports on zinc status in the Sri Lankan school children until we undertook the present study (4). However, folic acid status among adolescent girls and non-pregnant, non-lactating young women in Colombo has been studied and published recently (5) which showed that 43% had low serum folic acid concentrations and 47% had low iron stores with an anaemia prevalence of 12.9 %. Therefore, to combat these deficiencies a proper long term nutritional intervention is a necessity.

In addition to the National salt fortification programme, a fortification of wheat flour with iron was tested out in 1998-1999. Wheat flour fortified with 66mg/Kg of electrolytic or reduced iron was tested in a double blind, controlled trial in the estate sector of Sri Lanka with an aim to reduce the prevalence of anaemia (6). After two years of intervention neither electrolytic nor reduced iron had an effect on haemoglobin concentration among study subjects. The findings from this study suggested that fortification of wheat flour with iron was not beneficial in reducing anaemia in the population studied. Wheat, however is an imported food item and as such, not feasible for a long term national food fortification. We therefore, selected a locally produced cereal, the rice as a suitable vehicle for fortification.

In most parts of the world, the average diet is predominantly cereal, with wheat, rice and maize being the staples. In Asia, rice provides more than 70% of the energy in the diet (7). Rice is the single most important crop occupying 34 percent (0.77 million hectares) of the total cultivated area in Sri Lanka. About 1.8 million farm families are engaged in paddy cultivation island-wide. Sri Lanka currently produces 2.7 million tonnes of paddy annually and satisfies around 95 percent of the domestic requirement (8). Rice provides 45% of total energy and 40% of total protein requirement of an averageSri Lankan. As such, rice can be a good candidate for fortification with micronutrients because it is the staple diet of all sectors of the population, consumed almost daily in large amounts in Sri Lanka. The intervention programme was concerned with a rice flour fortification to increase the micronutrient intake and thereby to improve growth and status of iron, zinc and folate of Sri Lankan population was conducted with the approval from the Ethical Review Committee of the Faculty of Medicine.

Materials and Methods

Investigation I Survey on rice flour consumption pattern The rice, as flour is being used by Sri Lankans in the preparation of their meals. But no data are available on the level of consumption of rice flour *per se*. Therefore, a survey by using a pre-tested questionnaire to study the patterns of purchasing, consumption and acceptability of meals made out of rice flour in a representative sample from Galle district was conducted. The aim of this cross sectional suvey was to determine the actual intake of rice and rice flour based meals, as well as study factors that influence the dietary intake of rice flour.

A two-stage stratified random sampling design was used in the survey. Urban, semi-urban and rural households of the Galle district were the domains for stratification. Randomly selected Public Health Midwifery (PHM) areas (n=29) were the primary census blocks. The income and expenditure survey in 2002 carried out by Department of Census and Statistics, Sri Lanka (9), has calculated that a sample size of 1050 housing units would represent the Galle District. We randomly selected streets in each PHM areas and then all housing units on that street were included in the study. On average there were 35-38 households per street in the selected areas, so that a total of 1096 households were randomly selected for the survey.

The survey was implemented in March and April 2004 and the interviews were undertaken after obtaining written informed consent. A trained interviewer visited each house and either the head of the household or the spouse or both were interviewed. The questionnaire was used to obtain information on rice consumption pattern -especially the quantity of rice and rice flour purchased and utilized by the household during the preceding month. The ownership of the rice growing fields as well as meal preparation practices with rice flour in the household was also documented. The social class of each family was defined by a scoring system after considering the occupation, aggregate family income, educational status of chief house holder and the housing conditions (3). Social classes were classified as class 1 (cumulative score of 19-20); class 2 (cumulative score of 17-18); class 3 (cumulative score of 13-16); class 4 (cumulative sore of 9-12); and class 5 (cumulative score of 4-8). Intakes of rice and rice flour were calculated using following formula; *'amount consumed x frequency per day x (number of days per week/month)'*

Investigation II Fortification of rice flour and consumer acceptance of fortified rice flour based meals Rice flour was prepared at the Agro Food Technology (AFT) division of the Industrial Technology Institute (ITI), Colombo by grinding brown country rice (well polished 6-8 % weight reduction on polishing -Grade 2) to 300- 500 mesh size using an electric grinder. Because of established high bioavailability and proven efficacy (10), the ferrous sulfate and zinc oxide were selected as fortificants to be used in this study. Fortificant levels used were 60 mg of elemental mineral (i.e., iron, zinc) to 1 kilo gram of rice flour, based on guidelines adapted in other countries (11, 12). To overcome the inhibitory effects of phytates in rice, disodiumEDTA was included at a molar ratio of 1:1 of iron:EDTA, a ratio that has been shown to be both efficacious and safe in previous studies (13). Folic acid was added to achieve the concentration of 2 mg per kilo of rice flour and 162.85mg of dried ferrous sulfate ($FeSO_4$) and 74.12mg of zinc oxide (ZnO) powder were added to each kg of rice flour to obtain the desired level of fortification. In addition, 385.08mg of disodiumEDTA in dry powder form was added to each kg of rice flour in appropriate groups in order to achieve a molar ratio of 1:1 elemental Fe:disodiumEDTA and 0.7:1 of elemental zinc:disodiumEDTA. The fortificants, $FeSO_4$ and ZnO, were supplied by Dr. Paul Lohmann, Germany; disodiumEDTA by AKZO NOBEL, Netherlands and folic acid by Glaxo Welcome through their local agent Greenfields International, Sri Lanka. Blending the fortificants with rice flour was accomplished at the ITI using a ribbon blender. Iron and zinc levels in the fortified rice flour were measured by flame atomic absorption spectrometry (f AAS) in random samples of rice flour to confirm proper mixing.

Experimental meals were prepared at the Nutrition Research Laboratory, Department of Community Medicine, Faculty of Medicine, Galle where the fortified flour was stored at ambient condition. Temperature ranged from 30°-40° C and relative humidity on or above 80%. The meals made out of the fortified flour at baseline, and then at monthly intervals up to three months of storage, were subjected to sensory evaluation for taste, odour, acceptance and texture. Duplicate fortified and control flour (non-fortified) samples were tested by panelists (n=13), in the laboratory. The evaluation panel consisted of the volunteers from the staff (academic (n=3), non-academic (n=5) and students (n=5)) of Faculty of Medicine. They were convened to test one type of food per day on three days. On each day dishes made out of fortified flour of different storage levelsand the control flour were tested. Three commonly consumed rice flour dishes that were identified at the consumption survey i.e. *pittu*, string hoppers and *aluwa* were used.

Pittu is a flat small 'droplet' prepared by mixing rice flour with water and coconut and steamed on a pre-heated water pan. String hoppers are thin noodles made out of preheated rice flour and mixed with waer and then steamed. *Aluwa* is sweet cake made from rice flour mixed with tricle/ sugar and water, cooked and spreaded on a board. The formulae of the meals used in this study are the ones commonly practiced by Sri Lankan people.

The sensory evaluation was based on testing for multiple sample difference in quality attributes following the procedures used in India (14). The panelists evaluated the coded meals based on taste, colour, odour and acceptability, using a hedonic scale of -5 to +5, with zero being the value assigned to the control food made with unfortified flour. The panelists were also invited to make comments on the evaluation forms. In addition, the technicians who were responsible for preparing the meals kept records of their observations on the characteristics of the uncooked flour during their preparations.

Investigation III: Determination of the bioavailability of iron and zinc from folate-fortifed rice flour and study of the enhancing effect of disodium ethylene- diamine- tetraacetic-acid (disodiumEDTA) on bioavailability Children between the ages of 7-10 years were recruited for this study through a meeting of potential participants and their families from University field training area. Informed written consent was obtained from the parents after explaining in detail the purpose, risks and benefits of the study. Fifty seven (57) children came and 7 were excluded (3 parents refused phlebotomy;2 children were taking supplements; venous access was not possible in one child; and another child had a history of seizures). Based on a study for iron absorption from fortified wheat flour seen in a similar population in Indonesia (13) a sample of 18 was predicted to give greater than 90% power in detecting the assumed difference of about 8% between the $FeSO_4$ group and the $FeSO_4$+ disodiumEDTA group. Adding 6 more subjects to each group to accommodate drop-outs, the minimum requirement was 24 subjects in an arm. To assess the interaction of zinc with iron, each arm was divided into two resulting in four groups of investigation.

Arm 1 – Without EDTA
- **Group 1- $FeSO_4$ and folate (n=13);**
- **Group 2- $FeSO_4$, ZnO and folate (n=12);**

Arm 2 – With EDTA
- **Group 3 - $FeSO_4$, disodiumEDTA and folate (n=13);**
- **Group 4 - $FeSO_4$, disodiumEDTA, ZnO and folate (n=12).**

Stable isotopes that were used in this study (^{57}Fe, ^{58}Fe, ^{67}Zn and ^{70}Zn) were purchased from Trace Sciences International, Toronto, Canada. The ^{67}Zn and ^{70}Zn (90 % enrichment by mass) were obtained as zinc oxide dry powder, and prepared each in an aqueous solution of 0.085 mg per ml and then tested for sterility and pyrogenicity using the quantitative chromogenic limulus amebocyte lystate test (QCL-1000 kit from BioWhittaker, BioWhittaker Molecular Applications) at the Investigational Pharmacy of Texas Children's Hospital, Houston, Texas. ^{57}Fe (95% enrichment by mass) and ^{58}Fe (96% enrichment by mass) were provided in elemental metal form. Iron isotope solution for administration was prepared, as the sulfate, in our laboratory, by dissolving metals in 0.03 ml of 7M nitric acid and 0.125 ml of 0.5M sulfuric acid for every mg of elemental iron. The solutions were dried at 120°C, at 230°C, and finally at 500°C for 30 minutes each in a muffle furnace. After cooling, the final products were re-suspended in 0.2M sulfuric acid at 0.24 ml for every mg of iron. Deionized water was added to produce a solution yielding a unit dose of 1.5 mg elemental iron for each 2.5 ml solution.

Each test meal, '*halapa*', was individually prepared. Rice flour (fortified with folic acid only) was weighed on a calibrated scale within –0.1g of the desired weight (25g). Each portion of rice flour was mixed with 15mL of doubly distilled water and kneaded for 2-3 minutes until a smooth dough was produced. The dough was flattened on a leaf (*Macaranga peltata*) that is commonly used for flavoring in Sri Lanka. Next, 1.5mg of elemental iron in the form of $^{58}FeSO_4$ (all 4 groups), 9.627mg of disodiumEDTA (group 3 and 4) and 1.5mg of elemental zinc in the form of ^{67}ZnO (group 2 and 4) were spreaded over the dough. The exact dose was carefully measured and recorded with the subject's identification number. The amount of isotope added was in the same proportion of the elements present in the fortified flour. A mixture of grated coconut (12g) cooked in sugar syrup (10g) was spreaded on each of the dough, and then the *halapa* was folded and steamed for 10 minutes. The steamed product was stored in a refrigerator and heated in the microwave oven just before the administration of the meal. The levels of the iron and zinc in the preparations were confirmed by f-AAS, and the phytate level was measured by Association of Official Analytical Chemists (AOAC) method and found to be 22.5mg per *halapa*.

On day one, all children were asked to arrive at the testing site (Faculty of Medicine) at 0630 hours after fasting overnight except for water. A reference iron isotope dose that consisted of 5 mg ^{57}Fe as iron sulfate dissolved in orange juice with 50mg of ascorbic acid added was administered to them. Orange juice and ascorbic acid were used as enhancers of iron absorption. The children were asked to avoid any meal for two more hours to prevent any interaction with the contents of such a meal. On the following morning, again after an overnight fast, each subject was given a test meal according to one's group allocation. They fasted for an additional 2 hours and then resumed heir usual diet. Subsequently, a reference dose of ^{70}Zn was given intravenously to subjects of arms 2 and 4 who had been receiving a meal made out of zinc-fortified rice flour.

Approximately 72 hours after the test meal, 25mL of urine was collected in to urine collection bags from subjects in arms 2 and 4 to measure fractional excretion of zinc. Two children dropped out at this stage and as such, 48 children completed the trial. Fourteen days after the isotope administration a venous blood sample (5mL) was obtained from all and red cells were separated to measure red blood cell (RBC) iron isotope enrichment. Of the blood samples taken for measurement of ^{57}Fe and ^{58}Fe, three were contaminated during transportation. In addition, ^{58}Fe was not available to provide an adequate dose to two subjects. Therefore, 45 samples for ^{57}Fe, and 43 samples for ^{58}Fe were analyzed. Twenty four samples were evaluated for zinc absorption from zinc groups.

Iron isotope ratio was measured using a thermal ionization magnetic sector mass spectrometer (MAT 261; Finnigan ThermoQuest, Bremen, Germany). The results were expressed as the ratio of ^{58}Fe to non administered iron isotope i.e. ^{56}Fe. The ratio of two non-administered isotopes (^{56}Fe and ^{54}Fe) was used to correct for temperature-specific differences in fractionation. Iron absorption was calculated from iron incorporation, based on the assumption that 90% of the absorbed iron was incorporated into red blood cells. Urinary zinc isotope enrichments were measured similarly to iron isotopes. Isotope ratios were expressed with respect to a non-administered isotope (^{66}Zn) and corrected for differences in fractionation with the use of ^{64}Zn to ^{66}Zn ratio (two non administered zinc isotopes). Zinc absorption was calculated from the relative fractional excretion of the oral and intravenous isotope doses in 72-hour urine samples. All the isotope measurements were done at the Children's Nutrition Research center, Baylor College of Medicine, Houston, Texas, USA.

Investigation IV: Efficacy of fortified rice flour fortification in improving iron, zinc and folate status and anthropometry of children As the final step of this research programme, a pilot efficacy study over a period of one month was carried out as a prelude to a future placebo controlled study on efficacy of fortified rice flour in a Sri Lankan population sample. The subjects used for the absorption study were recruited for this study as well after obtaining informed written consent from their parents. The subjects were randomized into four groups based on type of fortification as mentioned in the previous investigation. The weight and the height were recorded and a medical history and a physical examination were performed on each subject before the fortified rice flour was given. All subjects were given 75g per day of fortified rice flour prepared according to their group allocation, to be consumed daily for a period of 4 weeks. Parents were instructed to prepare this as a common food item for the whole family (families were supplemented with fortified rice flour packets containing 75g/ per person/ day). A venous blood sample (5mL) was obtained from each subject for the determination of Hb and serum levels of ferritin, zinc and folate at the beginning and at the end of the trial and their weight and height of each was also recorded. Baseline and final biochemical and anthropometry parameters of the subjects were compared.

Results

Investigation I Of the total sample of 1096 families 314 were from urban communities, 485 represented semi-urban and 297 were from rural settings of the

Galle District (Table 1). The mean monthly consumption of rice per family was 36.5Kg. The mean rice consumption per person per meal observed in the different sectors in this study population was not significantly different from each other (137.71g in urban; 138.95g in semi-urban; 134.44g in rural; p=0.40). Subjects in the urban sector had significantly lower per capita rice flour consumption (130.3 g/meal; p=0.05) when compared with semi-urban (142.57 g/meal) and rural (148.67 g/meal) sectors. Rice flour consumption patterns by the social classes are illustrated in Table 2. Families of social class 2 had higher rice consumption than in the other social classes. But no such difference was observed in the intake of rice flour. 48.8% of families in class 1 and 67.8% of families in class 2 had purchased rice flour (Table 2) whereas only 18% of the lowest income group (class 5) purchased rice flour in the preceding month.

Average family size of the study sample was 4.1; majority of the families were of social classes 3 and 4. Overall, 86% of households consumed rice flour and two out of five households purchased rice flour from the market (Table 3). Urban (44.5%) and semi-urban (43.1%) households purchased rice flour, at a 15% higher rate than the rural (27.2%) sector (Table 3). Nearly half of the households (49.8%) bought readymade food items made out of rice flour. Common rice-based meals eaten were string hoppers, pittu, hoppers and sweet cakes. 12.8% of the study population had their own paddy fields; the majority belonging to rural communities.

Table 1 Rice flour consumption patterns according to sector[1]

		Average consumption in grams (mean, 95% CI)	
Sector	n	rice flour (person/day)	rice flour (family/day)
Urban	314	130.34 (121.8; 138.9) [a]	836.52 (782.3; 890.7)
Semi-urban	485	142.57 (134.1; 157.0) [b]	836.47 (797.0; 876.0)
Rural	297	148.67 (136.2; 161.1) [b]	897.52 (837.7; 957.3)
All	1096	140.72 (135.1; 146.3)	853.03 (824.6; 881.4)

[1] Results tabulated as mean and 95% confidence Interval
[a, b] values with same superscripts in a column are not significantly different ($p<0.05$)

Table 2 Rice and rice flour consumption patterns according to social class[1]

		Average consumption (grams)		% of families purchasing rice flour
	n	rice (person/meal) mean (95% CI)	rice flour (person/day) mean (95% CI)	
Class 1	41	131.45 (118.1-144.8)[a]	132.56 (117.7; 153.4)	48.8
Class 2	90	150.44 (139.4-161.5)[b]	134.91 (118.5; 151.3)	67.8
Class 3	567	136.95 (133.5-140.4)[a]	142.82 (135.0; 150.6)	39
Class 4	371	117.83 (131.9-142.0)[a]	141.99 (131.6; 152.4)	33.4
Class 5	27	137.37 (105.6-130.1)[a]	110.78 (92.2; 129.4)	18.5

[1] Groups with different superscript letters (a, b) different significantly ($p<0.05$)

Investigation II The external characteristics of cooked food items showed that all types of flour had similar properties to that of the control after one month of storage. Since the flour was made up of brown country rice there was no obvious darkness or discoloration in the fortified flour due to oxidation of ferrous sulfate. However, when the flour was stored for more than two months, black spots were observed

Table 3 Popularity of rice flour based food consumption according to area of living[1]

		Urban sector	Semi-urban sector	Rural sector	All
Families (n)		314	485	297	1096
Rice flour	-home made	174 (55.4%)	276 (56.9%)	216 (72.7%)	666 (60.8%)
	-buy	140 (44.6%)	209 (43.1%)	81 (27.3%)	430 (39.3%)
Rice flour consumption	Frequently	276 (87.9%)	416 (85.8%6)	251 (84.5%)	943 (86.0%)
Reasons for infrequent consumption	-price	10 (3.2%)	17 (3.5%)	16 (5.4%)	43 (3.9%)
	-taste	14 (4.5%)	33 (6.8%)	9 (3.0%)	56 (5.1%)
	-prep:	4 (1.3%)	6 (1.2%)	13 (4.4%)	23 (2.1%)
	-other	10(3.2%)	13 (2.7%)	8 (2.7%)	31 (2.8%)
Purchase of ready-made meals		173 (55.4%)	248 (51.1%)	125 (42.1%)	546 (49.8%)

[1] Results were expressed as number of families (percentage)

Table 4 Sensory attributes of meal[1]

	Product	Mean sensory value						
Sensory attribute		0 month		1 month		2 month		3 month
Acceptability[1]	S. Hoppers	1.41		1.06		0.53		0.31
	Pittu	2		1.44		1		0.92
	Aluwa	1.18		0.77		0.44		0.31
	P-value		*0.23*		*0.61*		*0.49*	
Texture	S. Hoppers	2.06		1.06		0.84		0.69
	Pittu	1.39		0.94		0.68		0.62
	Aluwa	1.53		1.12		1		0.82
	P-value		*0.23*		*0.6*		*0.08*	
Odour[2]	S. Hoppers	1.77		1.12		1.05		0.85
	Pittu	2.17		1.56		1.05		1.08
	Aluwa	0.94		0.53		0.63		0.69
	P-value		*0.14*		*0.11*		*0.21*	
Taste	S. Hoppers	1.41		1		0.74		0.46
	Pittu	2.17		1.44		0.95		0.92
	Aluwa	1.47		0.94		0.58		0.39
	P-value		*0.11*		*0.52*		*0.33*	

[1] There is a significant difference in acceptability of Pittu over Aluwa (p=0.028)
[2] There is a significant difference in odour of Pittu over Aluwa (p=0.009); and String hoppers (p=0.013); Meals of second month were significant from meals at the end of first month (p=0.004)

Table 5 Percentage iron absorption from the reference dose and the test dose

	Ref. dose	Test dose	P-value			
	(^{57}Fe) Absorption	(^{58}Fe) Absorption	Grp 1	Grp 2	Grp 3	Grp 4
Group 1	31.4 % – 11.7	2.5% – 1.5	---	0.23	0.32	0.03
Group 2	30.3 % – 12.8	3.5% – 2.0	0.23	---	0.03	0.1
Group 3	31.9 % – 08.0	1.9% – 1.1	0.32	0.03	---	0.005
Group 4	30.4 % – 12.6	6.1% – 4.4	0.03	0.09	0.005	---
Mean	31.0 % – 10.9	3.4 % – 2.9				
P-value	0.98	0.003				

[1] Derived from the Analysis of variance (ANOVA)

Table 6 The baseline anthropometry and serum biochemistry of the study subjects[1]

Parameter	Group 1	Group 2	Group 3	Group 4	Overall
Sex	M 6; F 8	M 7; F 6	M 6; F 7	M 6; F 7	M 25; F 28
Age (months)	88.0 – 18.7	94.9 – 19.5	94.8 – 19.2	100.9 – 15.9	94.1 – 18.4
Weight (Kg)	19.9 – 3.8	20.5 – 3.6	20.1 – 3.9	21.7 – 4.5	20.5 – 3.9
Height (cm)	119.2 – 7.3	122.3 – 9.2	121.0 – 8.2	124.5 – 9.3	121.8 – 8.5
BMI	13.9 – 1.7	13.6 – 0.9	13.6 – 1.3	13.8 – 1.5	13.8 – 1.4
Haemoglobin (g/dL)	11.9 – 1.1	12.2 – 0.9	12.3 – 1.2	12.2 – 1.1	12.2 –1.1
Serum Ferritin (g/L)	47.3 – 30.4	52.9 – 36.8	51.9 – 27.1	42.0 – 23.9	48.5 – 29.4
Serum Folate (nmol/L)	12.8 – 4.9	12.4 – 4.1	12.1 – 5.6	13.0 – 8.1	12.6 – 5.7
Serum Zinc (mol/L)	12.1 – 2.7	11.8 – 2.4	11.8 –2.3	12.1 – 1.7	11.9 – 2.2

[1] Group 1- (Fe+folate); group 2- (Fe+EDTA+folate); group 3- (Fe+Zn+folate); group 4- (Fe+EDTA+Zn+folate); results presented in mean – SD

Table 7 The anthropometry of the children following intervention[1]

Parameter		Group 1	Group 2	Group 3	Group 4	Overall
Weight (Kg)	Pre	19.9 – 3.8	20.5 – 3.6	20.1 – 3.9	21.7 – 4.5	20.5 – 3.9
	Post	19.9 – 2.7	20.7 – 3.0	20.9 – 4.3	22.0 – 4.7	20.9 – 3.7
	P-value	0.01	0.03	<0.001	<0.001	<0.001
Height (cms)	Pre	119.2 – 7.3	122.0 – 9.2	121.0 – 8.2	124.5 – 9.3	121.8 – 8.5
	Post	120.7 – 7.0	122.4 – 8.2	122.2 – 8.0	125.1 – 9.8	122.6 – 8.2
	P-value	<0.001	<0.001	<0.001	<0.001	<0.001
BMI	Pre	13.9 – 1.7	13.6 – 0.9	13.6 – 1.3	13.8 – 1.5	13.8 – 1.4
	Post	13.6 – 0.9	13.7 – 0.9	13.7 – 1.4	13.9 – 1.5	13.8 – 1.2
	P-value	0.2	0.26	0.04	0.003	<0.001

[1] Group1-(Fe+folate); group2- (Fe+EDTA+folate); group3- (Fe+Zn+folate); group4- (Fe+EDTA+Zn+folate); results presented in mean – SD; p-value from the paired t-test comparing pre and post intervention

Table 8 The effect on haematology following intervention[1]

Parameter		Group 1	Group 2	Group 3	Group 4	Overall
Hb (g/L)	Pre	119.9 – 11.3	121.9 – 9.8	123.1 – 12.2	121.9 – 11.1	121.7 – 10.9
	Post	125.0 – 07.8	127.0 – 10.3	129.8 – 06.3	128.6 – 08.3	127.6 – 8.2
	P-value	0.64	0.11	0.02	0.008	<0.001
Ferritin (g/L)	Pre	47.3 – 30.4	52.9 – 36.8	51.9 – 27.1	42.0 – 23.9	48.5 – 29.4
	Post	48.5 – 25.1	55.4 – 32.1	49.5 – 21.2	46.8 – 35.4	50.0 – 28.1
	P-value	0.93	0.55	0.76	0.59	0.66
Folate (nmol/L)	Pre	12.8 – 4.9	12.4 – 4.1	12.1 – 5.6	13.0 – 8.1	12.6 – 5.7
	Post	17.5 – 3.6	19.4 – 9.6	17.4 – 6.3	21.3 – 9.9	18.8 – 7.7
	P-value	0.03	0.02	0.02	<0.001	<0.001
Zinc (mol/L)	Pre	12.1 – 2.7	11.8 – 2.4	11.8 – 2.3	12.1 – 1.7	11.9 – 2.2
	Post	12.3 – 2.8	12.1 – 2.2	13.0 – 2.5	12.7 – 2.6	12.5 – 2.5
	P-value	0.61	0.56	0.15	0.27	0.06

Group 1-(Fe+folate); group2- (Fe+EDTA+folate); group3- (Fe+Zn+folate); group4- (Fe+EDTA+Zn+folate); results presented in mean – SD; p-value from the paired t-test comparing pre and post intervention in string hoppers and *aluwa*. There were no obvious signs of surface dehydration (stiff-textured or sticky in consistency) that could result in the products.

All sensory characteristics of foods remained positive during the whole period of flour storage (Table 4). Nevertheless, compared with the baseline the acceptability declined over storage time although there was no consistency in the direction of change. A significant difference in acceptability of *pittu* over *aluwa* (p=0.03) was observed during the course of the trial whereas there was no difference between acceptance of *aluwa* over string hoppers (p= 0.79) and *pittu* over string hoppers (p= 0.05). There was no difference in acceptability with the duration of flour storage (p= 0.434). Texture of the meals did not show any significant differences over the type of food (p=0.43), or the duration of storage (p=0.31). Compared with the control meals there was a significant difference in the odour of *pittu* over *aluwa* (p=0.009) and string hoppers (p=0.01). A significant difference in odour between the first month and the second month was seen in all 3 meals (p=0.004). There was no significant difference in taste between the type of meals (p=0.99) and duration of storage (p=0.74).

Investigation III Iron absorption from the reference dose of ^{57}Fe was similar among the 4 groups, with a mean of 31.0% (Table 5). The overall absorption of ^{58}Fe from the meal in all groups was 3.4 ±2.5% (geometric mean of 2.8%). Significant intra-group differences were seen for absorption of ^{58}Fe. The highest fractional absorption of Fe from the meal was in group 4 (6.1±4.4%) and lowest in group 3 (1.9±1.1%; Table 5).

The presence of zinc did not adversely affect absorption of ^{58}Fe from the meal. In fact, the absorption was greater in subjects groups 3 and 4 who received zinc (3.8 ±3.7%) than in groups 1 and 2 who did not receive zinc (3.0 ±1.8%) although this difference was not statistically significant (p>0.10). Absorption of ^{58}Fe from a meal was significantly greater (p<0.05) in groups 2 and 4 (4.7 ±3.6%) than those in groups 1 and 3 (2.2 ±1.3%). However, when group data were combined, those who consumed disodiumEDTA (groups 2 and 4) and those who did not (groups 1 and 3), there was a significant negative interaction between zinc and disodiumEDTA. In the groups not consuming zinc (groups 1 and 2), there was no significant difference in iron absorption (p<0.10) with or without disodiumEDTA. Among the groups that consumed meals containing zinc (groups 3 and 4), a highly significant increase (p=0.005) was seen in group 4 that was given disodiumEDTA.

In a multivariate model of fractional absorption of ^{58}Fe included the group, initial ferritin and haemoglobin levels, age, sex, and weight, only the group (p<0.01) and sex (p=0.02) were significant. Males (4.4 ±3.7%) had a higher fractional absorption than females (2.5 ±1.5%). Based on the iron content in the fortified rice flour (60mg/kg), the absorption of added iron was highest in group 4 (6.1 ±4.4%). Children in this group had average absorption of 92µg (±66.3 µg) of iron from the 25g of rice flour in the test meal. This represents about 13% (range, 4-22%) of the estimated absorbed requirement (0.7 to 0.8mg) of iron in this age group (15).

Fractional zinc absorption averaged 10.9 ±5.1% overall in those groups who received a meal made with zinc-fortified rice flour. Fractional absorption of zinc was 13.5 ±6.0% in group 4 and 8.8 ±2.0% in group 3. The difference between the two groups was statistically significant (p= 0.04). In a multivariate model to predict fractional zinc absorption including the group, initial zinc level, sex, age, and weight; only the group was statistically significant (p= 0.04). Average absorption of added zinc in the meal was 202µg (±89.8 µg) in group 4 and 132µg (±30µg) in group 3 respectively. This constitutes approximately 17% (±8.0%) and 11% (±2.5%) of the 1200 µg of recommended absorbed zinc needs of an 8 year old child (16).

Investigation IV The sample consisted of 25 male and 28 female subjects with an age range of 7 to 10 years. There were no significant differences between subgroups in terms of age, anthropometrics and biochemical data (Hb, ferritin, zinc, and folate) at the beginning of the study (Table 6). At baseline 21% were underweight and 17.0 % were stunted. Further, baseline data from this study showed that 38.0% of the subjects were anaemic, 8.0% had low SF, 36.0% were deficient in folate and 15.0% had zinc deficiency. statistically significant improvement in anthropometry was observed with the intervention from their respective baseline value (Table 7, p<0.001). However, BMI was not significantly improved in groups 1 and 2. The zinc supplemented groups (groups 3 & 4) had a mean weight gain of 780g whereas non-zinc treatment groups (groups 1 & 2) had only 570g. These values were not significant (p= 0.205). Haemoglobin and serum folate levels significantly improved (p<0.001) during the intervention (Table 8). When the sub-group analysis was made, all four groups had significant improvement in serum folate from their respective baseline value (overall 6.28nmol/L, 6.28nmol/L, $p<0.001$). Groups 3 and 4 had shown a significant improvement in Hb concentration ($p=0.01$). Overall, levels of SF and serum zinc improved (by 1.51µg/L and 0.59µmol/L respectively) during this short period of time, but these changes were not significant ($p=0.10$). In the groups that received disodiumEDTA when compared with those that did not receive a greater increase in SF and Hb in the EDTA groups was evident. However, the increased was statistically significant in Hb only ($p=0.03$). The groups that received zinc-fortified meals showed a gain in serum zinc, but did not reach a statistically significant level.

Discussion

Food fortification provides maximum benefit for minimum investment (17) and is generally recognized as being the most efficient as well as the most cost-effective means of eliminating micronutrient deficiencies when compared with supplementation. The results of fortification are broad and sustainable when compared with supplementation. A staple food that is consumed regularly by the majority of the population is generally used as a vehicle for fortification and as such, high population coverage can be easily achieved.

The observed monthly per family consumption of rice in our study is close to the finding (35.3Kg) of the national survey (9). According to the national survey 70% of households spent more than half of their total expenditure for food and drink in Sri Lanka and *per capita* rice flour consumption of 70.75g with 296.45g per household. However, our findings are of double this amount (140.7g). This could be due to the fact that our results are based not only on rice flour purchased but also on home made as well. The social class difference in rice flour purchasing patterns explains, in part, that there is an avenue to popularize fortified rice flour and food products of fortified flour among poorer segments of the population. We did not quantify the common rice flour based food products that the national survey had identified. However, in our study we found that string hoppers, hoppers and *piitu* were the most frequently consumed rice flour based food items in Sri Lanka. Urban households appear to have better access to prepared rice-based foods; hence a lesser quantity of rice flour based foods are prepared at home. Rural households (72%) prefer home-made rice flour. It may be due to poor access for ready-made flour and prepared food outlets and also due to cultural reasons. Small proportions of households claimed that rice flour based meals are not tasty (5.0%), or high in cost (3.9%) and need longer preparation time (2.1%).

There are some theoretical as well as practical advantages on using rice flour to deliver micronutrients to children through complementary feeding. It is unlikely that other kinds of flour especially wheat would be readily available to children of low-income families at a low cost in our country if the government discontinues the subsidiary programme on wheat. On the other hand it is the rice that is being used as the first cereal in the preparation of complementary foods for babies by the Sri Lankan mothers. The findings of this survey show that rice flour appears to be an appropriate vehicle for fortification with micronutrients. Since most of home-made complementary foods in Sri Lanka are also based on either rice or rice flour, our findings suggest that toddlers too can benefit from such fortification.

It was evident during this consumer acceptance trial that the quality of flour did not get deteriorated with time although rancidity of ferrous sulfate was reported in a previous study on wheat flour fortification in Sri Lanka (18), where wheat flour fortified with sodiumironEDTA and ferrous fumarate became less acceptable sooner than the flour fortified with reduced iron and electrolytic iron. But in our trial as we did a multiple micronutrient fortification i.e. ferrous sulfate with disodiumEDTA, zinc oxide and folic acid, its' sensory characteristics could have been different from the previous wheat flour trial. The effect of rancidity/ discoloration of ferrous sulfate may have been masked by the presence of disodiumEDTA (19). The quality of flour although supposed to be deteriorated with time, the taste, texture and acceptability remained within the desired limits at the third month of storage as well. The ambient flour storage conditions in Sri Lanka are harsh, with high temperatures and humidity. Flour under such conditions requires relatively rapid consumption. The results obtained by this investigation confirm that fortified rice flour stored upto a period of 3 months was accepted by the subjects for preparation of their meals. It can be concluded that the odour, acceptance, taste and texture of meals made out of rice flour with 60 mg/kg fortification level of iron and zinc had satisfactory sensory attributes and therefore, enrichment of rice flour could be considered as a feasible strategy in Sri Lanka.

This is the first-ever trial in Sri Lanka examining the absorption of micronutrients from the fortified rice flour. The fractional absorption of both iron and zinc from fortified rice flour was lower than predicted.

However, it was evident that disodiumEDTA enhanced the absorption of both micronutrients. The rice flour used in this study had relatively low phytate concentrations (i.e. 110±10 mg/100g). The phytic acid content in the local rice is variety-dependent (250 to 530 mg/100g with an average of 320 mg/100g), and polishing (6-8% in our rice) will further reduce the phytate to a range of 80 to 330 mg/100g (20). The phytic acid:zinc molar ratio of this study was 15:1. The low fractional absorption in this study can be attributed solely to high phytate levels. However, the zinc status of the individuals also needs to be considered. Zinc absorption increases with increasing zinc deficiency (16). The relative adequacy of the zinc status of the population studied may have contributed to the low bioavailability of zinc observed.

Analysis of the results demonstrated that regular consumption of micronutrient-fortified rice flour for four weeks led to improvements in weight, height and several measures of micronutrient status in semi urban school children in Galle. The improved growth (weight and height) in the study groups cannot be assumed to be due to a higher energy intake from the rice flour itself, as a control group was not included in the study. But there are other potential explanations. One of the cardinal features of zinc deficiency is growth failure; thus, it is possible that the increase in zinc intake augmented lean tissue accretion or increased utilization of the energy provided. Such a possibility is described in the response of BMI in this study as it significantly improved only in zinc supplemented groups. Secondly, it is possible that it stimulated an increase in appetite of the children and thereby increasing their energy intake. This could have resulted in the improvement in growth and general status of health of these children. Thirdly, improvement in iron stores, and enhanced serum folate and zinc status *per se* may have had an impact in limiting morbidity, which in turn can lead to improvement in growth and development of children.

The mean improvement in haemoglobin was higher in zinc supplemented groups. Thus, it was demonstrated that the added zinc had no deleterious effect on iron stores. The increase in serum zinc and folate concentrations observed at the end of the study strongly suggests a beneficial effect on zinc and folate status of primary school children as a result of consumption of the fortified rice flour. This is of great importance as improvement in iron, zinc and folate status have been shown to enhance growth and decrease morbidity and mortality from infectious diseases in school children in developing countries (21).

The present pilot efficacy study is limited by the small size of the study population. Further, the duration of supplementation may not be sufficient to draw definite conclusions on the effectiveness of the fortification formula used. It is not possible to definitively evaluate the outcomes without a control group, although our results are likely to reflect the true benefit of the fortified rice flour. Therefore, a definitive randomized, placebo-controlled, double blind study covering multiple age groups over an extended period should be conducted in order to evaluate the effectiveness of the proposed rice flour fortification before implementing at national level.

Conclusions

In this research programme, rice flour was evaluated as a potential vehicle for fortification with micronutrients such as iron, zinc and folate. One of the limiting factors in fortifying a dietary staple is the lack of simple and affordable technology to fortify with stable and bioavailable nutrients without compromising commonly accepted taste and appearance. A meal made out of 50g of fortified rice flour, one fourth of the daily recommended absorbed iron and zinc content of a primary school child can be met. In order to evaluate efficacy and effectiveness of rice flour fortification, a definitive randomized, placebo-controlled, double blind studies in multiple age groups over an extended period is necessary. Therefore multi-sector approach to be adopted in the establishment of any food-fortification programme, encompassing the participation of relevant governmental organizations, food industry, trade organizations, consumers, academic and research facilities, marketing specialists and any involved international organizations and agencies is recommended.

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References

1. Hurrel RF. Fortification: Overcoming Technical and Practical Barriers. J Nutr 2002;132:806S-812S
2. Department of Health Services Annual Health Bulletin- 1999. Ministry of Health, Sri Lanka. 113 pp

3. Ministry of Health. Assessment of anaemia status in Sri Lanka. Survey report by MRI. 2003. Department of Health Services. Sri Lanka
4. Hettiarachchi M. Liyanage C. Wickremasinghe R. et al. Prevalence and severity of micronutrient deficiency: a cross - sectional study among adolescents in Sri Lanka. *Asia Pac J Clin Nutr 2006;* 15(1):56-63
5. Thoradeniya T. Wickremasinghe R. Ramanayake R. et al. Low folic acid status and its association with anaemia in urban adolescent girls and women of childbearing age in Sri Lanka. *Bri J Nutr* 2006:95(3):511-516
6. Nestel P. Nalubola R. Sivakaneshan R. et al. The use of iron-fortified wheat flour to reduce anemia among the estate population in Sri Lanka. *Int J Vit Nutr Res* 2004:74(1):35-51
7. Garrow LS. James WP. Human nutrition and dietetics. 9th ed. Churchill Livingstone
8. USDA FAS report 2004 - Sri Lanka. USA
9. Household Income and Expenditure Survey -2002. Final Report. Household Income and Expenditure Survey. Department of Census and Statistics. Sri Lanka
10. FAO. Technical Consultation on food fortification: technology and quality control. Rome. Italy 1995
11. The Micronutrient Initiative. Guidelines for Iron Fortification of Cereal Food Staples. Sharing US Technology to Aid in the Improvement of Nutrition. Washington. DC 2001
12. Rosado JL. Zinc and Copper: Proposed Fortification leverls and recommended zinc coumpounds. *J Nutr* 2003:133:2985S-2989S
13. Herman S. Griffin IJ. Suwarti S. et al. Co-fortification of iron fortified flour with zinc sulfate but not zinc oxide. decreases iron absorption in Indonesian children. *Am J Clin Nutr* 2002:76:813-817
14. Indian standards. Indian Standards No 6273. part II. New Delhi. India
15. Institute of Medicine. Iron. In: DRI (Dietary Reference Intake). National Academy Press. pp. 290
16. Institute of Medicine. Zinc. In: DRI (Dietary Reference Intake). National Academy Press. pp. 442
17. Nilson A. Piza J. Food Fortification: A tool for fighting hidden hunger. Food and Nutrition Bulletin 1998;19(1):49-60 17
18. Goonaratne J. Mudalige R. Purvis G. Product evaluation using Iron fortified wheat flour. *Cey J Med Sci* 1996:39:23-34
19. Hurrell RF. Preventing iron deficiency through food fortification. *Nutrition Review* 1997:55:210–222
20. Hapuarachchi S. Gooneratne J. Kumarapperuma SC. Effect of parboiling on the reduction of phytate in different varieties of rice. SLAAS Proceedings of the 59th Annual Session- Part I – 604E2 (abs) 2003
21. Maberly GF, Trowbridge FL, Yip R, et al. Programs against micronutrient malnutrition: ending hidden hunger. *Annual Review of Public Health* 1994:15:277-301