

Iron absorption from fish sauce and soy sauce fortified with sodium iron EDTA¹⁻³

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ABSTRACT

Background: Fish sauce and soy sauce have been suggested as food vehicles for iron fortification in Asia. NaFeEDTA is a potentially useful fortificant because it can be added to these condiments without causing precipitation during storage.

Objectives: The objectives were to evaluate iron absorption from NaFeEDTA-fortified fish sauce and soy sauce against a reference fortificant (FeSO₄), to compare iron absorption from NaFeEDTA-fortified fish sauce and soy sauce, and to evaluate the influence of fish sauce and soy sauce per se on iron absorption.

Design: Five separate iron-absorption studies were made in adult women (10 women per study). Iron absorption was measured on the basis of erythrocyte incorporation of ⁵⁷Fe or ⁵⁸Fe 14 d after the intake of labeled meals of rice or rice and vegetables. Fish sauce or soy sauce (10 g) fortified with 5 mg Fe as NaFeEDTA or FeSO₄ was fed with selected meals. The results are presented as geometric means.

Results: Iron absorption from NaFeEDTA- and FeSO₄-fortified fish sauce (3.3% and 3.1%, respectively) and soy sauce (6.1% and 5.6%, respectively) was not significantly different. No significant difference was observed when NaFeEDTA-fortified fish sauce and soy sauce were compared directly (6.7% and 7.9%, respectively). Soy sauce inhibited iron absorption from rice-based meals (8.5% without and 6.0% with soy sauce; *P* < 0.02), whereas fish sauce did not affect iron absorption significantly.

Conclusion: The relatively high iron absorption from NaFeEDTA-fortified fish sauce and soy sauce and the acceptable organoleptic properties of NaFeEDTA indicate the potential usefulness of this iron fortificant in fish sauce and soy sauce fortification programs. *Am J Clin Nutr* 2003;78:274-8.

KEY WORDS Iron, absorption, stable isotopes, soy sauce, fish sauce, NaFeEDTA, iron fortification

INTRODUCTION

Food-fortification programs are usually considered the most cost-effective and sustainable approach to combating iron deficiency (1, 2). In Southeast Asia, where the prevalence of iron deficiency (including the more severe form of iron deficiency, iron deficiency anemia) is high (3), the major staple food, rice, would seem the obvious choice as the iron-fortification vehicle. Unfortunately, rice is difficult to fortify because it is not usually consumed as flour. Attempts have been made to fortify rice with iron by coating rice grains with iron (4-6) or by adding iron-fortified simulated rice grains (7). Further development of these

technologies, however, is needed before any large-scale evaluations can be made.

When fortification of a staple food is not feasible, condiments are useful alternatives as fortification vehicles. Fish sauce is a condiment frequently used in Southeast Asia. For example, 80% of the population in Vietnam regularly consumes fish sauce (8). In Southeast Asian cuisine, this clear brown liquid—manufactured by fermenting freshwater or saltwater fish with salt (9, 10)—is the equivalent to salt in the West. Another condiment that is frequently consumed in Asian countries is soy sauce. In China, soy sauce has been used as an all-purpose seasoning for thousands of years. It is produced by the yeast fermentation of soybeans and wheat or of soybeans alone (11).

Besides the advantage of being consumed at a relatively high rate daily, soy sauce could be especially useful as a fortification vehicle because it has been shown to enhance iron absorption from rice (12). The reason for this reported enhancing effect is unclear because soy products are known to inhibit iron absorption because of their high phytic acid content and because of the peptides formed during digestion of the conglycinin (7S) fraction of soy protein (13, 14). Phytic acid, however, can be expected to be substantially degraded during the fermentation process, and the soy proteins can be expected to be transformed into a mixture of amino acids and peptides that might enhance iron absorption. The effect of fish sauce on iron absorption has not been studied.

The aim of the present study was to evaluate iron absorption from NaFeEDTA-fortified fish sauce and soy sauce. NaFeEDTA, an iron compound that was recently approved for use in supervised food-fortification programs (15), was evaluated because preliminary studies have indicated that it is the most promising iron-fortification compound for fish sauce and soy sauce because it produces no off-flavors, off-colors, or precipitation during storage. Iron absorption from rice-based meals fed with NaFeEDTA-fortified sauces was compared with iron absorption from meals fortified with ferrous sulfate in the same women. The influence of

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the sauces per se on iron absorption from rice was evaluated and, last, a direct comparison of iron absorption from rice meals fortified with NaFeEDTA-fortified fish sauce or with NaFeEDTA-fortified soy sauce was made. Iron absorption in young women was estimated on the basis of the incorporation of stable isotopes into erythrocytes 14 d after administration.

SUBJECTS AND METHODS

Subjects

Fifty apparently healthy women (aged 19–29 y; maximum body weight 60 kg) were recruited from the student and staff populations of the Swiss Federal Institute of Technology (Zurich, Switzerland) and the University of Zurich. The subjects were randomly allocated to 5 separate studies (10 subjects per study). Exclusion criteria included pregnancy or lactation and known gastrointestinal or metabolic disorders. No medication (except oral contraceptives) or vitamin or mineral supplements were allowed during the study. Women regularly taking vitamin or mineral supplements discontinued supplementation 2 wk before the start of the study.

The study protocol was reviewed and approved by the Ethical Committee of the Swiss Federal Institute of Technology. Subjects were informed orally and in writing about the aims and procedures of the study. Written informed consent was obtained from all study subjects.

Study design

Iron absorption was determined with the use of a double stable-isotope technique. The iron compounds were labeled with ^{57}Fe or ^{58}Fe and added to the different test meals as described below. All test meals were fed, after the subjects had fasted overnight, on 2 consecutive days under standardized conditions. Iron absorption was based on erythrocyte incorporation of ^{57}Fe or ^{58}Fe 14 d after intake of the labeled test meals. A crossover study design was used, with each woman acting as her own control. On day 1, a venous blood sample was drawn after an overnight fast for determination of iron-status indexes (hemoglobin, ferritin, and circulating transferrin receptor). Body weight and height were measured, and then the first labeled test meal was served. On the following day, the second test meal was administered. Test meals were fed under strictly standardized conditions under close supervision. No intake of food or fluids was allowed for 3 h after the intake of the test meals. A second venous blood sample was drawn 14 d after intake of the second test meal (day 16).

Test meals

All test meals included boiled white rice (50 g dry wt, Jasmine perfume rice; Dragon Phoenix Brand, Bangkok, Thailand). In studies 1 and 2, the rice was fed with vegetables (44% Chinese cabbage, 22% carrots, 22% zucchini, and 13% onions), which were boiled until tender and then stir-fried in vegetable oil before being puréed (25 g fresh wt/serving). The vegetable purée was prepared in bulk and kept frozen until consumed. Fish sauce (10 g, Cat Hai medium-quality fish sauce; Cat Hai, Hai Phong, Vietnam) or soy sauce (10 g, Wadakan soy sauce; Beijing Hetiankuan Food Company, Xishanqi, Beijing) were added to some of the test meals (see below). The sauces were purchased in bulk in Vietnam and China, respectively. Each test meal contained 5 mg added Fe; 4 mg as labeled ferrous sulfate or NaFeEDTA plus 1 mg Fe of normal isotopic composition. Deionized water (200 g) was served as a drink.

In study 1, rice plus vegetable purée were served with 10 g fish sauce fortified with $\text{Na}^{58}\text{FeEDTA}$ or $[^{57}\text{Fe}]$ ferrous sulfate. In study 2, rice plus vegetable purée were served with 10 g soy sauce fortified with $\text{Na}^{58}\text{FeEDTA}$ or $[^{57}\text{Fe}]$ ferrous sulfate. The test meals in study 3 consisted of rice only, which was fortified with $[^{57}\text{Fe}]$ or $[^{58}\text{Fe}]$ ferrous sulfate; 10 g fish sauce was added to one of the meals. Study 4 was identical to study 3 except that it was served with 10 g soy sauce. In study 5, 10 g fish sauce fortified with $\text{Na}^{58}\text{FeEDTA}$ or soy sauce fortified with $\text{Na}^{57}\text{FeEDTA}$ was served with rice alone.

Stable-isotope labels

Stable-isotope labels ($[^{57}\text{Fe}]$ ferrous sulfate and $[^{58}\text{Fe}]$ ferrous sulfate) were prepared from isotopically enriched elemental iron (Chemgas, Boulogne, France) by dissolution in sulfuric acid. The solutions were stored in polytetrafluoroethylene containers flushed with argon to keep iron in the 2^+ oxidation state. $\text{Na}^{58}\text{FeEDTA}$ and $\text{Na}^{57}\text{FeEDTA}$ were prepared in solution from ^{58}Fe - and ^{57}Fe -enriched elemental iron. The metal was dissolved in 1 mL (^{58}Fe) or 2 mL (^{57}Fe) HCl, diluted with water, and stored in polytetrafluoroethylene containers. The resulting FeCl_3 solution was mixed with a freshly prepared aqueous Na_2EDTA solution ($\text{Na}_2\text{EDTA} \cdot 2\text{H}_2\text{O}$; Sigma Chemical Co, St Louis) at a molar ratio of 1:1 (Fe:EDTA) and added to individual servings of fish sauce or soy sauce (10 g) 22–24 h before the test meals were administered. The containers were wrapped in aluminum foil and kept refrigerated overnight.

Quantification of iron isotopes in labels

Isotope-dilution mass spectrometry was used to measure iron concentrations of the labeled compounds in solution. An accurately measured amount of iron of natural isotopic composition was added to aliquots taken from the prepared isotopic labels. The iron standard was prepared gravimetrically from an isotopic reference material (IRM-014; EU Institute of Reference Materials, Geel, Belgium). Isotopic analysis was performed with the use of negative thermal ionization–mass spectrometry (16). Iron concentrations in the isotopic labels were calculated on the basis of the shift in iron isotopic abundances, the determined isotopic abundances of the pure isotopic labels, and the natural iron isotopic abundances (17).

Iron-status measurements

Venous blood samples (7 mL) were drawn into EDTA-treated tubes before the first labeled test meal was consumed and again on day 16. Samples were analyzed for iron-status indexes (hemoglobin, plasma ferritin, and circulating transferrin receptor) and for the incorporation of ^{57}Fe and ^{58}Fe into erythrocytes (day 16). Blood samples were portioned for the measurement of hemoglobin, and plasma was separated, portioned, and frozen for later measurement of ferritin and circulating transferrin receptor concentrations. Hemoglobin was measured with the use of the cyanmethemoglobin method (Sigma kit; Sigma Chemical Co); plasma ferritin and circulating transferrin receptor concentrations were measured with the use of enzyme-linked immunosorbent assay (Ramco Laboratories, Houston). Commercial quality-control materials (DiaMed, Cressier sur Morat, Switzerland, and Ramco Laboratories) were analyzed together with samples analyzed for hemoglobin and plasma ferritin, respectively.

Quantification of iron isotope in blood

Each isotopically enriched blood sample was analyzed in duplicate for its iron isotopic composition as previously described by Davidsson et al (18). The blood samples were mineralized by



TABLE 1
Contents of iron, calcium, phytic acid, and ascorbic acid in the test meals¹

Meal	Iron		Calcium	Phytic acid ²	Ascorbic acid
	Fortification	Native			
	mg		mg	mg	mg
Study 1: rice, vegetables, fish sauce	5	0.4	16.2	25	0.4
Study 2: rice, vegetables, soy sauce	5	0.9	16.0	27	0.4
Studies 3 and 5: rice, fish sauce	5	0.2	5.8	25	ND
Studies 4 and 5: rice, soy sauce	5	0.7	5.6	27	ND
Studies 3 and 4: rice	5	0.1	2.0	25	ND

¹ND, not determined and assumed to be negligible.

²The molar ratio of phytic acid to iron was 0.4:1 in all meals.

using a mixture of nitric acid and hydrogen peroxide and microwave digestion. The iron was separated from the matrix by anion-exchange chromatography and a solvent-solvent extraction step into diethylether. The isotopic analyses were performed by negative thermal ionization–mass spectrometry (16).

Calculation of iron absorption

The amounts of ⁵⁷Fe and ⁵⁸Fe isotopic labels in blood 14 d after administration of the test meals were calculated on the basis of the shift in iron isotope ratios and on the amount of iron circulating in the body. The calculations were based on the principles of isotope dilution and took into account that iron isotopic labels were not monoisotopic (17). Circulating iron was calculated on the basis of blood volume and hemoglobin concentration (19). Blood volume calculations were based on height and weight according to Brown et al (20). For calculations of fractional absorption, 80% incorporation of the absorbed iron into erythrocytes was assumed (21).

Food analysis

The iron and calcium contents of rice, vegetable purée, fish sauce, and soy sauce were measured with the use of electrothermal flame atomic absorption spectroscopy (SpectrAA 400; Varian, Mulgrave, Australia) after mineralization by microwave digestion (MLS 1200; Microwellen Labor System, Leutkirch, Switzerland) in a mixture of HNO₃ and H₂O₂ and with the use of the standard addition technique to minimize matrix effects. The amount of phytic acid in rice was measured with HPLC (22, 23), and the phytic acid content in soy sauce was measured with the use of the Makover (24) method, which was modified by replacing iron with cerium in the precipitation step. Ascorbic acid in the vegetable purée was measured by HPLC with a reversed-phase column and photometric detection (Merck-Hitachi System, Merck, Germany) (25, 26). The nitrogen content of the fish sauce and soy sauce was measured by Kjeldahl analysis, and the amino acid content was measured by ion-exchange chromatography (27)

Statistics

Absorption ratios are presented as geometric means (–1 SD, +1 SD). Student's paired *t* test was used to compare absorption data within each study. Absorption values were logarithmically transformed before statistical analysis (EXCEL 97; Microsoft Corporation, Redmond, WA).

RESULTS

Subjects

Two of the 50 women had iron-deficiency anemia (hemoglobin: 119 and 118 g/L; transferrin receptor: > 8.5 mg/L; and

plasma ferritin: < 12 μg/L). Sixteen women had iron deficiency on the basis of either an elevated transferrin receptor concentration (> 8.5 mg/L), a low plasma ferritin concentration (< 12 μg/L), or both.

Test meals

The contents of iron, calcium, phytic acid, and ascorbic acid in the different test meals are shown in **Table 1**. The total iron content varied between 5.1 and 5.9 mg/meal. Most of the iron (5 mg) came from the fortification compound; smaller amounts came from soy sauce (0.7 mg) and fish sauce (0.2 mg), puréed vegetables (0.1 mg), and rice (0.1 mg). The calcium content was low and varied between 2 and 16 mg/meal. Nearly all phytic acid came from the rice (25 mg/meal). Soy sauce contained 20 mg phytic acid/100 g but provided only 2 mg phytic acid/meal. The phytic acid content of the vegetable purée was not measured and was assumed to be negligible. The molar ratio of phytic acid to iron in the test meals was ≈0.4:1. There was little ascorbic acid remaining in the cooked and puréed vegetables. The ascorbic acid content of all meals was negligible. The nitrogen contents of the fish sauce and soy sauce were 1.3 and 1.1 g/100 g, respectively, and the amino acid contents after hydrolysis were 4.0 and 5.3 g/100 g, respectively.

Iron absorption

The geometric mean iron absorption from the different test meals varied between 3.1% and 11.6% (**Table 2**). There was no significant difference in iron absorption from the rice and vegetable meal served with fish sauce fortified with ferrous sulfate or NaFeEDTA (study 1: geometric \bar{x} , 3.1% compared with 3.3%; *P* = 0.66) and from the same meal served with soy sauce fortified with ferrous sulfate or NaFeEDTA (study 2: geometric \bar{x} , 5.6% compared with 6.1%; *P* = 0.44). Fish sauce per se did not influence iron absorption from rice (study 3: geometric \bar{x} , 9.5% compared with 11.6%, with and without sauce, respectively; *P* = 0.14), although the consumption of soy sauce per se resulted in a significant decrease in iron absorption (study 4: geometric \bar{x} , 6.0% compared with 8.5%, with and without sauce, respectively; *P* = 0.02). When compared directly, there was no significant difference in iron absorption from the rice meal fed with NaFeEDTA-fortified fish sauce and that fed with NaFeEDTA-fortified soy sauce (study 5: geometric \bar{x} , 6.7% compared with 7.9%; *P* = 0.08).

DISCUSSION

Iron absorption from meals fed with NaFeEDTA-fortified fish sauce was not significantly different from that from identical test

TABLE 2

Iron absorption by healthy adult women from rice-based meals served with iron-fortified fish sauce or soy sauce or without any added condiment¹

Meal	Iron status		Iron absorption					P ²
	Ferritin	Transferrin receptor	FeSO ₄ -fortified fish sauce	NaFeEDTA-fortified fish sauce	FeSO ₄ -fortified soy sauce	NaFeEDTA-fortified soy sauce	FeSO ₄ -no sauce	
	µg/L	mg/L			%			
Study 1: rice and vegetables	23 (10, 50)	7.6 (6.2, 9.4)	3.1 (1.2, 7.9)	3.3 (1.6, 6.8)	—	—	—	0.66
Study 2: rice and vegetables	14 (9, 23)	8.5 (6.7, 10.8)	—	—	5.6 (3.3, 9.5)	6.1 (3.2, 11.8)	—	0.46
Study 3: rice	16 (7, 34)	7.5 (1.2, 9.2)	9.5 (4.3, 21.0)	—	—	—	11.6 (5.6, 23.8)	0.14
Study 4: rice	16 (9, 29)	6.1 (5.4, 6.9)	—	—	6.0 (2.3, 15.8)	—	8.5 (3.8, 19.1)	<0.02
Study 5: rice	17 (8, 36)	8.5 (6.0, 12.1)	—	6.7 (3.7, 12.1)	—	7.9 (4.7, 13.5)	—	0.08

¹Geometric \bar{x} (−1 SD, +1 SD); $n = 10$ women per study.²The statistical analysis was conducted within each study by paired t test.

meals fed with ferrous sulfate–fortified fish sauce. Nor was there a statistical difference between iron absorption from meals fed with NaFeEDTA-fortified soy sauce and the identical meals fed with ferrous sulfate–fortified soy sauce (Table 2). Although iron absorption from the 2 iron fortificants evaluated in the current study was similar, it is important to stress that major differences exist in relation to provoking unacceptable organoleptic changes. NaFeEDTA can be added to fish sauce and soy sauce without changing the sensory properties of the fortified condiment (28), whereas the addition of ferrous sulfate causes unacceptable precipitation (M Fidler, unpublished observations, 2002).

Although iron absorption from NaFeEDTA has been shown to be 2–3 times higher than ferrous sulfate from meals with a high phytic acid content, no significant difference in the iron absorption of the 2 iron compounds from moderately inhibitory meals was reported (29). It was therefore not surprising that in the present study NaFeEDTA had no enhancing effect on iron absorption relative to ferrous sulfate. The phytic acid content of the rice meals fed in the present study was 25–27 mg at an iron content of 5.1–5.9 mg (Table 1). The molar ratio of phytic acid to iron ($\approx 0.4:1$) was low and can be considered as only moderately inhibitory to iron absorption (30). The results of the present study indicate a relatively high iron absorption from the rice meals containing NaFeEDTA-fortified fish sauce or NaFeEDTA-fortified soy sauce and that these sauces are potentially useful as iron-fortification vehicles. After adjustment of the iron-absorption values (Table 2) to a serum ferritin concentration corresponding to no iron stores, 12 µg/L (31), it is estimated that iron absorption would be in the range 5.9–15.2% (\bar{x} : 9.9%) from rice meals accompanied by these NaFeEDTA-fortified sauces. For example, if the sauces were fortified with 0.5 mg Fe/mL and the consumption was 20 mL/d, the sauces would provide 10 mg extra dietary Fe. The fortified condiment would thus provide $\approx 30\%$ of the daily iron requirements of young women based on an iron absorption from the diet of 10% (type 2 diet) (32).


On the basis of previous studies, it was somewhat unexpected that soy sauce and fish sauce per se did not enhance iron absorption from the rice meals. Soy sauce was previously reported to enhance iron absorption from a rice meal (12), and fish and a mixture of amino acids similar to that in fish protein have been reported to improve iron absorption from a variety of meals (33–35). In the present study, the addition of soy sauce decreased iron absorption by $\approx 30\%$ ($P = 0.02$; study 4; Table 2), whereas fish sauce had no significant effect on iron absorption (study 3; Table 2). However, no significant difference between iron absorption from rice meals fed

with NaFeEDTA-fortified fish sauce and that from NaFeEDTA-fortified soy sauce was found in the same women ($P > 0.05$; study 5).

Several different types of commercial soy sauce are currently manufactured, and the contradictory effects of soy sauce on iron absorption between the present study and that of Baynes et al (12) can presumably be explained by differences in the composition of the sauces (ie, variations in raw ingredients) and by differences in manufacturing methods. In China, the traditional soy sauce, which is comparable with Japanese tamari-shoyu, is prepared from soybeans only, whereas the modern type of soy sauce is made from defatted soybean meal and wheat bran (36, 37). The soy sauce used by Baynes et al was the tamari-shoyo type, whereas the Chinese soy sauce used in the present study was the modern type, which included wheat bran as a raw ingredient. Tamari-shoyo sauces are subjected to a longer fermentation time than are the modern type sauces, which results in a greater extent of hydrolysis and, therefore, a larger proportion of amino acids and low-molecular-weight peptides in the final product (36). These differences in composition could be important when evaluating iron absorption because the extent of protein hydrolysis might influence the amount of inhibiting soy protein fractions present in soy sauce (14). In support of this hypothesis, Macfarlane et al (38) showed, with few exceptions, that there is an inverse relation between iron absorption and the proportion of higher-molecular-weight fractions in the soy proteins. It is unlikely that the inhibition of iron absorption by phytic acid was the reason for the inhibitory effects of soy sauce in the present study, because the very small amounts of phytic acid in the soy sauce used in this study (2 mg/test meal) would not be expected to significantly influence iron absorption (13).

The nitrogen content is a determinant of the quality of fish sauce. The supernatant fluid collected after the first fermentation is referred to as high-quality fish sauce. Lower-quality fish sauces are produced by extracting the fermented fish residue with hot brine. These lower-quality sauces contain less nitrogen and consequently smaller amounts of amino acids and peptides (10). In the present study, a medium-quality fish sauce was evaluated because this is the quality of sauce most frequently consumed in Vietnam. Thus, the low content of amino acids and peptides in the Vietnamese fish sauce used in the present study may have been the reason why it did not enhance iron absorption. The amount of fish sauce (10 g) added to the test meals contributed only 0.4 g amino acids and peptides. This amount is considerably lower than the amount of fish protein (≈ 10 –20 g) that has been shown to enhance iron absorption (33–35) in a dose-dependent manner (35).

Finally, although fish sauce is made from the fermentation of whole fish, including the bones, the calcium content of the Vietnamese fish sauce was low (4 mg/10 g) and was not expected to influence iron absorption (39, 40).

In conclusion, the results of the 5 studies show that iron absorption by young women consuming rice meals served with NaFeEDTA-fortified fish sauce or NaFeEDTA-fortified soy sauce is relatively high and that both NaFeEDTA-fortified sauces appear to be useful vehicles for iron fortification. 

MCF, LD, TW, and RFH contributed to the study design. MCF and LD implemented the study. TW prepared the stable isotope labels and performed the analytic work. LD conducted the statistical analysis. MCF and LD wrote the manuscript and TW and RFH edited the manuscript.

RFH is a member of the Technical Advisory Board of ILSI Project IDEA (Iron Deficiency Elimination Action). At the time of the study, LD was a member of the Scientific Advisory Committee, ILSI Project IDEA. None of the other coauthors reported any conflict of interest.

REFERENCES

- Hurrell RF. Preventing iron deficiency through food fortification. *Nutr Rev* 1997;55:210–22.
- Hurrell RF. Improvement of trace element status through food fortification: technological, biological and health aspects. *Bibl Nutr Dieta* 1998;54:40–57.
- World Health Organization. National strategies for overcoming micronutrient malnutrition. Geneva: WHO, 1992. (Draft resolution proposed by reporters of the Executive Board, 89th Session.)
- Salcedo J, Bamba MD, Carrasco EO, et al. Artificial enrichment of white rice as a solution to endemic beriberi. A report of field trials in Bataan, Philippines. *J Nutr* 1950;42:501–23.
- Florentino R. Iron fortification studies in the Philippines. Proceedings of the annual meeting of the International Nutritional Anemia Consultative Group (INACG). New York: The Nutrition Foundation, 1981. (Section V.)
- Peil A, Barrett F, Rha C, Langer R. Retention of micronutrients by polymer-coatings used to fortify rice. *J Food Sci* 1982;47:260–2.
- Kapanidis AN, Lee TC. Novel method for the production of color-compatible ferrous sulfate-fortified simulated rice through extrusion. *J Agric Food Chem* 1996;44:522–5.
- Mannar V, Gallego EB. Iron fortification: country level experiences and lessons learned. *J Nutr* 2002;132:856S–8S.
- Newman JM. Cultural aspects of Asian dietary habits. In: Ang CY-KW, Huang Y-W, Liu K, eds. *Asian foods: science and technology*. Boca Raton, FL: CRC Press, 1999:453–85.
- Lopetcharat K, Choi YJ, Park JW, Daeschel MA. Fish sauce products and manufacturing: a review. *Food Rev Int* 2001;17:65–88.
- Liu K. Oriental soyfoods. In: Ang CY-KW, Huang Y-W, Liu K, eds. *Asian foods: science and technology*. Boca Raton, FL: CRC Press, 1999: 139–99.
- Baynes RD, Macfarlane BJ, Bothwell TH, et al. The promotive effect of soy sauce on iron absorption in human subjects. *Eur J Clin Nutr* 1990;44:419–24.
- Hurrell RF, Juillerat MA, Reddy MB, Lynch SR, Dassenko SA, Cook JD. Soy protein, phytate, and iron absorption in humans. *Am J Clin Nutr* 1992;56:573–8.
- Lynch SR, Dassenko SA, Cook JD, Juillerat MA, Hurrell RF. Inhibitory effect of a soybean-protein-related moiety on iron absorption in humans. *Am J Clin Nutr* 1994;60:567–72.
- Joint FAO/WHO Expert Committee on Food Additives (JECFA). Fifty-third meeting, Rome: FAO/WHO, 1999. Internet: <http://www.who.int/pcs/jecfa/summary53revised.pdf> (accessed 2 June 2003).
- Walczyk T. Iron isotope ratio measurements by negative thermal ionization mass spectrometry. *Int J Mass Spectrom Ion Processes* 1997;161:217–27.
- Walczyk T, Davidsson L, Zavaleta N, Hurrell RF. Stable isotope labels as a tool to determine iron absorption by Peruvian school children from a breakfast meal. *Fresenius J Anal Chem* 1997;359:445–9.
- Davidsson L, Walczyk T, Zavaleta N, Hurrell R. Improving iron absorption from a Peruvian school breakfast meal by adding ascorbic acid or Na₂EDTA. *Am J Clin Nutr* 2001;73:283–7.
- Kastenmayer P, Davidsson L, Galan P, Cherouvrier F, Hercberg S, Hurrell RF. A double stable isotope technique for measuring iron absorption in infants. *Br J Nutr* 1994;71:411–24.
- Brown E, Hopper J Jr, Hodges J Jr, Bradley B, Wennesland R, Yamauchi H. Red cell, plasma and blood volume in healthy women measured by radiochromium cell-labelling and hematocrit. *J Clin Invest* 1962;41:2182–90.
- Hosein F, Marsaglia G, Finch CA. Blood ferrokinetics in normal man. *J Clin Invest* 1967;49:1–9.
- Sandberg A-S, Ahderinne R. HPLC method for determination of inositol tri-, tetra-, penta-, and hexaphosphates in foods and intestinal contents. *J Food Sci* 1986;51:547–50.
- Sandberg A-S, Carlsson N-G, Svanberg U. Effects of tri-, tetra-, penta-, and hexaphosphates on in vitro estimation of iron availability. *J Food Sci* 1989;54:159–61.
- Makower RU. Extraction and determination of phytic acid in beans (*Phaseolus vulgaris*). *Cereal Chem* 1970;47:288–95.
- Sapers GM. Determination of ascorbic acid, dehydro-ascorbic acid and ascorbic acid-2-phosphate in infiltrated apple and potato tissue by HPLC. *J Chromatogr* 1990;503:431–6.
- Parviainen MT, Nyssönen K. In: De Lenheer A, Lambert WE, Nelis HJ, eds. *Modern chromatography analysis of vitamins*. New York: Marcel Dekker, 1992:235–60.
- Lebet V, Schneider H, Arrigoni E, Amado R. Kritische Beurteilung der Proteinbestimmung nach der Methode von Kjeldahl aufgrund der Aminosäureanalyse. (A critical appreciation on the protein determination by Kjeldahl based on the amino acid analysis.) *Mitt Gebiete Lebensm Hyg* 1994;85:46–58 (in German).
- Garby L, Areekul S. Iron supplementation in Thai fish-sauce. *Ann Trop Med Parasitol* 1974;68:467–76.
- MacPhail AP, Patel RC, Bothwell TH, Lamparelli RD. EDTA and the absorption of iron from food. *Am J Clin Nutr* 1994;59:644–8.
- Hurrell R. How to ensure adequate iron absorption from iron-fortified food. *Nutr Rev* 2002;60:S7–15.
- Cook JD, Dassenko SA, Lynch SR. Assessment of the role of nonheme-iron availability in iron balance. *Am J Clin Nutr* 1991;54:717–22.
- WHO/FAO. Recommended nutrient intakes. *Nutr Rev* (in press).
- Martinez-Torres C, Layrisse M. Effect of amino acids on iron absorption from a staple vegetable food. *Blood* 1970;35:669–82.
- Cook JD, Monsen ER. Food iron absorption in human subjects. III. Comparison of the effect of animal proteins on nonheme iron absorption. *Am J Clin Nutr* 1976;29:859–67.
- Bjorn-Rasmussen E, Hallberg L. Effect of animal proteins on the absorption of food iron in man. *Nutr Metab* 1979;23:192–202.
- Fukushima D. Fermented vegetable protein and related foods of Japan and China. *Food Rev Int* 1985;1:149–209.
- Liu K. Soybeans: chemistry, technology, and utilization. New York: Chapman & Hall, 1997.
- Macfarlane BJ, van der Riet WB, Bothwell TH, et al. Effect of traditional oriental soy products on iron absorption. *Am J Clin Nutr* 1990; 51:873–80.
- Cook JD, Dassenko SA, Whittaker P. Calcium supplementation: effect on iron absorption. *Am J Clin Nutr* 1991;53:106–11.
- Hallberg L, Brune M, Erlandsson M, Sandberg AS, Rossander-Hulten L. Calcium: effect of different amounts on nonheme- and heme-iron absorption in humans. *Am J Clin Nutr* 1991;53:112–9.