Iron absorption from fish sauce and soy sauce fortified with sodium iron EDTA\textsuperscript{1–3}

Meredith C Fidler, Lena Davidsson, Thomas Walczyk, and Richard F Hurrell

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 (FeSO\textsubscript{4}), 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 \textsuperscript{57}Fe or \textsuperscript{58}Fe 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 FeSO\textsubscript{4} was fed with selected meals. The results are presented as geometric means.

Results: Iron absorption from NaFeEDTA- and FeSO\textsubscript{4}-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; \textit{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.


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

\textsuperscript{1}From the Laboratory for Human Nutrition, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, Zurich, Switzerland.

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\textsuperscript{3}Reprints not available. Address correspondence to L Davidsson, Laboratory for Human Nutrition, Institute of Food Science and Nutrition, Swiss Federal Institute of Technology, PO Box 474, Seestrasse 72, 8803 Rueschlikon, Switzerland. E-mail: lena.davidsson@ilw.agrl.ethz.ch.

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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 $^{57}\text{Fe}$ or $^{58}\text{Fe}$ 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 $^{57}\text{Fe}$ or $^{58}\text{Fe}$ 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 pureéd (25 g fresh wt/serve). The vegetable pureéd 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 Heitiankuan Food Company, Xishanxi, 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 Na$^{58}\text{Fe}$EDTA or $^{57}\text{Fe}$ferrous sulfate. In study 2, rice plus vegetable purée were served with 10 g soy sauce fortified with Na$^{58}\text{Fe}$EDTA or $^{57}\text{Fe}$ferrous sulfate. The test meals in study 3 consisted of rice only, which was fortified with $^{57}\text{Fe}$ or $^{57}\text{Fe}$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 Na$^{58}\text{Fe}$EDTA or soy sauce fortified with Na$^{57}\text{Fe}$EDTA 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. Na$^{58}\text{Fe}$EDTA and Na$^{57}\text{Fe}$EDTA were prepared in solution from $^{58}$Fe- and $^{57}$Fe-enriched elemental iron. The metal was dissolved in 1 mL (50 Fe) or 2 mL (50 Fe) HCl, diluted with water, and stored in polytetrafluoroethylene containers. The resulting FeCl$_3$ solution was mixed with a freshly prepared aqueous Na$_2$EDTA solution (Na$_2$EDTA $\cdot$H$_2$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...
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 $^{57}$Fe and $^{58}$Fe 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 nitric acid and hydrogen peroxide and with the use of the standard addition technique (24). 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 with the use of the standard addition technique to minimize matrix effects. The amount of phytic acid in rice was measured by ion-exchange chromatography (27) +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 test meals are shown in Table 1. The total iron content varied between 5.1 and 5.9 mg/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/mg meal. Nearly all phytic acid came from the rice (25 mg/mg meal). Soy sauce contained 20 mg phytic acid/100 g but provided only 2 mg phytic acid/mg 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 fortified 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 meals.
5.1–5.9 mg (Table 1). The molar ratio of phytic acid to iron fed in the present study was 25–27 mg at an iron content of active to ferrous sulfate. The phytic acid content of the rice meals study NaFeEDTA had no enhancing effect on iron absorption rel-

ival of the 2 iron compounds from moderately inhibitory meals was be 2–3 times higher than ferrous sulfate from meals with a high

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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 pre-

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meals containing NaFeEDTA-fortified fish sauce or NaFeEDTA-fortified soy sauce and that these sauces are potentially useful as 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 soy-

beans 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-shoyu type, whereas the Chi-

nese 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 hydrolys-

sis and, therefore, a larger proportion of amino acids and low-
molecular-weight peptides in the final product (36). These differ-

ces in composition could be important when evaluating iron absorption because the extent of protein hydrolysis might influ-

ence 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 inhibi-

tion 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.

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