ETH
Eidgenössische Technische Hochschule Zürich
Swiss Federal Institute of Technology Zurich

IRON AND ZINCFORTIFICATION OF RICE: MEASURING BIOAVAILABILITY IN ORDER TO SET OPTIMAL FORTICATION LEVELS TO ENSURE PROGRAM EFFICACY

PRELIMINARY STUDY END REPORT

VERSION	1
DATE	25.11.2015
SPONSOR	GAIN, Global Alliance for Improved Nutrition Rue de Vermont 37 – 39 CH-1202 Geneva Switzerland
PRINCIPAL INVESTIGATOR	Dr. Diego Moretti, Laboratory of Human Nutrition, ETH Zürich, Schmelzbergstrasse 7 8006 Zürich

EXECUTIVE SUMMARY

Rice fortification has been proven to be feasible and efficacious. As a staple food for over 3 billion people worldwide, it can reach populations in both developing and industrialized countries. However, rice fortification is challenging. It is consumed as intact grains and fortification with iron as it can have an influence on organoleptic properties. The compound of choice for rice fortification is ferric pyrophosphate (FePP), which is water-insoluble and therefore typically has low bioavailability.

We conducted a series of studies *in vivo* and *in vitro* to optimize iron fortification of rice in terms of iron bioavailability and sensory acceptability.

Through the simultaneous extrusion of FePP with citric acid/trisodiumcitrate (CA/TSC), the iron bioavailability could be enhanced almost up to the level of $FeSO_4$, while still keeping an acceptable sensory profile. Our in vitro investigations did not show a cut-off for iron solubility, suggesting that increasing the CA/TSC ratios in the extruded rice could even result in higher bioavailability than measured in the human study. However, the addition of CA is limited due to sensory changes of the CA/TSC when added at high amounts, as could be shown in our colorimetric assessments.

Besides finding an optimal iron absorption enhancer, we also investigated the effect of zinc on iron bioavailability. It has been suggested, that zinc can negatively influence iron absorption, depending on the concentration of both minerals and the food fortification matrix. We found that iron absorption from FePP fortified rice was lower, when zinc oxide (ZnO) was used as zinc fortification compound rather than zinc sulfate ($ZnSO_4$). Both zinc compounds show advantageous sensory properties, but additional tests on the storage stability of iron and zinc fortified rice are suggested. Furthermore, it remains unclear whether the use of CA/TSC would counteract the inhibitory effect of ZnO on iron absorption.

To optimize all steps involved in rice fortification, not only the fortification compounds, but also the fortification technology was evaluated. We investigated iron bioavailability from FePP-fortified rice when produced under hot or cold extrusion conditions. Our findings show a doubled iron bioavailability in humans from cold extruded rice compared to hot extruded rice. Further investigations at our laboratory will complete these investigations by assessing iron and zinc bioavailability from hot extruded and coated rice.

We screened a range of potential novel iron fortification compounds for rice and, overall, our results suggest FePP remains the compound of choice for rice fortification, despite its low bioavailability. However, with the findings from our investigations, iron absorption from FePP can be significantly enhanced.

Our findings are based on single meal administrations of rice intrinsically labelled with stable iron isotopes, ensuring high precision and accuracy when assessing bioavailability. However, the approach of testing iron bioavailability in a single meal may have some limitations. Single meal imposes a cost limitation on a realistic kernel premix- natural rice ratio and in our studies we were only able to test 1:25 blending ratios of extruded kernel : natural rice ratios. A multiple meal study would allow testing 1:100 extruded kernel : natural rice ratios or similar, which are widely applied fortification ratios for rice.

SYNOPSIS

Iron and Zinc fortification of rice: measuring bioavailability in order to set optimal fortification levels to ensure program efficacy
Study acronym: Rice_FeZnHC
Date of protocol: 25.11.2015
Protocol number at local ethics committee: KEK-ZH-Nr. 2015-0212
Sponsor: GAIN, Global Alliance for Improved Nutrition Rue de Vermont 37 – 39 CH 1202 Geneva; Switzerland
Principal Investigator: Dr. Diego Moretti
Study type: Clinical trial with laboratory investigations (in vitro bioavailability and pilot plant trials)
Study site: Human Nutrition Laboratory, ETH Zürich
Primary objectives: 1) assessing whether iron bioavailability differs from hot or cold iron fortified extruded rice; 2) assessing iron solubility of extruded fortified rice; 3) assessing sensory properties of different iron compounds and absorption enhanders in extruded fortified rice
Secondary objectives: assessing the relative iron bioavailability in humans from fortified rice types
Study design: Single blind, randomized, cross-over design, with laboratory in vitro investigations and pilot plant trials of rice extrusion.
Methodology: 1) Iron bioavailability study with stable iron isotopic labels measured at least 14 days after test meal administration in red blood cells; 2) in vitro solubility trials; 3) Production of extruded rice prototypes and instrumental evaluation (color measurements)
Number of subjects recruited for human study: 20 Number of subjects who finished all procedures: 19

Diagnosis and main criteria for inclusion in the human study:

Inclusion criteria: Healthy (self-report), non-pregnant, female, age between 18 and 45, BMI between 18.5 to 25 kg/m^2

Exclusion criteria: pregnancy, lactation, iron infusion within last 12 months, presence of metabolic/ gastrointestinal/ kidney/ chronic diseases (e.g., diabetes, hepatitis, hypertension, cancer, cardiovascular diseases), long-term use of medication (with the exception of contraceptives), consumption of mineral and vitamin supplements within 2 weeks prior to 1st meal administration, blood transfusion/ donation or significant blood loss over the past 4 months, earlier participation in iron isotope study or participation in any study within 30 days prior to screening, non-compliance with the study protocol, smoking, vegan diet, drug/alcohol abuse.

Median duration of treatment: 45 days

Study period:	03.08.2015 – 02.10.2015
(date of first enrolment):	03.08.2015
(date of last completed):	02.10.2015

Criteria for evaluation

Efficacy: Fractional iron absorption and relative iron bioavailability of ferric pyrophosphate from iron fortified rice.

Safety: No adverse events were expected from the administration of iron in form of stable isotopes.

Statistical methods: All data will be converted to their logarithms for statistical analysis and reconverted for reporting. The fractional iron absorption from the different meals within the same participant will be compared by repeated-measures ANOVA followed by Bonferroni corrected pairwise comparisons. Differences are considered as significant at $P < 0.05$.

Summary

Iron absorption in nineteen apparently healthy women from two different rice meals was tested. The two meals both contained extruded rice with ferric pyrophosphate (^{57}FePP), zinc oxide (ZnO) and micronutrients and were fortified under hot (Meal FePP_H) or cold extrustion (Meal FePP_C) conditions.

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Abbreviations

AE	Adverse event
CA	Citric acid
CRF	Case report form
FAFe	Fractional iron absorption
FAFeS	Fractional iron solubility
FePP	Ferric pyrophosphate
$FeSO_4$	Ferrous sulfate
GCP	Good Clinical Practice
HFG	Humanforschungsgesetz (Law on human research)
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH)
KEK	Ethics Committee of the Canton of Zurich
MGFP	Micronized ground ferric pyrophosphate
NaEDTA	Natrium edetate
NaPP	Natriumpyrophosphate
PI	Principal Investigator
RP	Retinyl palmitate
SAE	Serious adverse event
SOP	Standard operating procedure
SUSAR	Suspected unexpected serious adverse reaction
TiO_2	Titanium dioxide
TSC	Tri-sodium citrate
USAID	United States Agency for International Developement
WFP	World Food program
ZnO	Zinc oxide
$ZnSO_4$	Zinc sulfate

1 STUDIES CONDUCTED UNDER THE GAIN RESEARCH GRANT

The following report presents the activities conducted under contract 99GL25-ML, aimed at assessing “Iron and Zinc fortification of rice: measuring bioavailability in order to set optimal fortification levels to ensure program efficacy”.

The study consisted of three main activities: 1) Assessing the impact of the extrusion technology on iron bioavailability from extruded, fortified rice, comparing cold (pasta-like process) to hot extrusion 2) Based on results indicating substantial increase of iron bioavailability when a mixture of Citric acid and trisodium citrate was coextruded with ferric pyrophosphate in extruded fortified rice (see 1.1), a detailed evaluation of its addition in different ratios and ranges was conducted in vitro, assessing iron solubility 3) To investigate alternative iron compounds for iron fortification of rice, a range of novel so far non-investigated iron fortification compounds was used to produce prototypes of extruded iron fortified rice and sensory properties (colour) were investigated instrumentally.

Our findings from a human absorption study are therefore accompanied with in vitro data from iron solubility experiments using differing molar ratios of citric acid and trisodium citrate (CA/TSC), which has shown to allow doubling iron bioavailability in humans. These investigations are reported with additional color measurements of extruded iron fortified rice kernels, whereas different iron compounds along with absorption enhancers were screened regarding their potential visual acceptability in rice.

During the duration of this research grant several other studies with wide programmatic impact have been conducted at our laboratory, for which the GAIN research grant has been instrumental in allowing establishing capacity and technical facilities for their conduction. The study outlines and results are summarized below. These projects were co-funded by DSM (Citric acid/Trisodiumcitrate study), PATH (Effect of zinc on iron bioavailability) and USAID/WFP (Iron and zinc bioavailability from coated and extruded rice).

1.1 Effect of Citric acid/Trisodium citrate on iron bioavailability from FePP fortified, extruded fortified rice

1.1.1 Background

Ferric phosphate (FePP) compounds are the only compounds that are widely used to fortify rice but their bioavailability in humans is known to be low. The aim of this study was to investigate a novel approach to assess whether the addition of a citric acid/trisodiumcitrate mixture (CA/TSC) prior to extrusion increases iron absorption in humans from FePP-fortified extruded rice grains.

1.1.2 Methods, subjects and results

We conducted an iron absorption study in 20 young women using four different meals (4 mg iron/meal): 1) extruded FePP-fortified rice (No Ca/TSC); 2) extruded FePP-fortified rice with CA/TSC added prior to extrusion (CA/TSC extruded); 3) extruded FePP-fortified rice with CA/TSC-solution added prior to consumption (CA/TSC solution); and 4) non-extruded rice fortified with a ferrous sulfate ($FeSO_4$) solution prior to consumption. Iron absorption was measured as erythrocyte incorporation of stable iron isotopes. Fractional iron absorption was significantly higher from meals CA/TSC extruded (3.2%) compared to meals No CA/TSC (1.7%) and CA/TSC solution (1.7%; all $P < 0.05$) and was not different from the $FeSO_4$ meal (3.4%; n.s.).

1.1.3 Conclusions and impact

Iron absorption from fortified rice is increased when FePP and CA/TSC are extruded simultaneously, most likely due to an *in situ* generation of soluble FePP-citrate moieties whose formations are based on the physical proximity and mixing of FePP and CA/TSC in extruded rice kernels. Due to the higher iron bioavailability when CA/TSC are added to rice, the overall fortification level in rice could be reduced, which would have a positive impact on the cost of fortification and on the sensory acceptability, when iron and zinc co-fortification is done using ZnO or $ZnSO_4$. Based on these results, the specifications of fortified rice for WFP food aid have been modified to contain a lower amount of iron and mention the need to add a chelating agent to the rice fortification premix (WFP technical specification RICE-FORTIFIED, version 15.0, 16.4.2015).

1.2 The effect of zinc on iron bioavailability from FePP fortified rice

1.2.1 Background

Rice is typically fortified with a mix of nutrients including zinc and iron. Interactions between iron and zinc bioavailability have been reported to occur under certain conditions. Evidence is limited as to how enhancers and inhibitors affect iron absorption from FePP, the compound of choice for rice fortification. Futhermore, for rice fortified with a premix approach, typically 1:100, only 1 % of the kernels are fortified with a much higher local concentration compared to other fortification vehicles. The aim of this study was to investigate whether co-fortification of rice either with ZnO or $ZnSO_4$ would affect iron bioavailability. As rice is typically fortified with both Fe and Zn the choice for the zinc compound has high programmatic importance as it may affect bioavailability, iron fortification levels and, in consequence, fortification costs.

1.2.2 Methods, subjects and results

We conducted an iron absorption study in 19 young women of low iron status (PF<16.5 µg/l) using four different meals (4 mg iron/meal): 1) extruded FePP-fortified rice (NoZn); 2) rice extruded with FePP and ZnO (FeZnO); 3) rice extruded with FePP and $ZnSO_4$ ($FeZnSO_4$); and 4) non-fortified extruded rice with a ferrous sulfate ($FeSO_4$) solution added prior to consumption. Iron absorption was measured as erythrocyte incorporation of stable iron isotopes 14 days after administration. Fractional iron absorption was significantly higher from meal $FeZnSO_4$ (4.5%) compared to meal FeZnO (2.7%); iron absorption in both cases did not differ significantly from NoZn (4.0%; n.s.).

1.2.2 Conclusion and impact

Iron absorption in iron deficient women from extruded fortified rice is lower when ZnO is used as fortification compound compared to $ZnSO_4$. This study suggests that zinc co-fortification with ZnO may affect the bioavailability from FePP. Whether the addition of a chelator such as CA/TSC would conteract the negative effect of ZnO on iron bioavailability requires further investigation.

1.3 Comparing iron and zinc bioavailability from coated and extruded fortified rice

1.3.1 Background

Limited evidence exists on the biological impact of rice coating, an alternative fortification technology to extrusion, which has been more extensively investigated in the literature. The aim of this study is to assess whether coated rice is comparable to extruded rice in terms of retention and absorption of micronutrients in human subjects.

1.3.2 Hypothesis

Comparing rice fortified via coating technology or hot extrusion, there will be no significant differences in micronutrient retention and cooking losses, or iron and zinc bioavailability in human subjects as measured with stable isotope techniques.

1.3.3 Outlook

Results of this study will be available in the public sphere (peer reviewed scientific literature) in the second half of the year 2016.

2 THE EFFECT OF HOT AND COLD EXTRUSION ON THE BIOAVAILABILITY FROM IRON FORTIFIED RICE

2.1 Ethics

2.1.1 Independent Ethics Committee or Institutional Review Board

The study protocol was submitted to the Ethics Committee of the Canton of Zurich (KEK Zurich) on March 25th 2015 for formal approval. All comments of the committee were replied to on July 17th 2015. The complete study protocol (KEK-ZH-Nr. 2014-0508) was approved on July 23rd 2015. The regular end of the study was reported to the KEK Zurich within 90 days, the final study report will be submitted within one year after study end.

2.1.2 Ethical Conduct of the Study

The study followed relevant government regulations and ETH research policies and procedures. The study has been conducted in accordance with principles enunciated in the current Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brazil, October 2013), the guidelines of Good Clinical Practice (GCP) issued by ICH, and Swiss regulatory authority's requirements. The formal consent of a participant, using the consent form approved by the KEK Zurich, was obtained before enrolment. This consent form had to be signed and dated by the participant and the investigator-designated research professional obtaining the consent.

2.1.3 Subject Information and Consent

The investigators explained to each participant the nature of the study, its purpose, the procedures involved, the expected duration, the potential risks and benefits. Each participant was informed that the participation in the study was voluntary and that she may withdraw from the study at any time without giving a reason. The participant was informed that her study records may be examined by authorized individuals other than the main investigator. All participants received a participant information sheet and a consent form describing the study and providing sufficient information for the participant to make an informed decision about her participation in the study. Participants were given enough time (approximately 1 week) to decide whether to participate or not. The participants were informed that they would be monetarily compensated for their participation (90 CHF).

The patient information sheet and the consent form were submitted to the KEK Zurich to be reviewed and approved. The formal consent of a participant, using the approved consent form, was obtained before the participant was submitted to any study procedure. The participant was asked to read and consider the statement before signing and dating the informed consent form, and was given a copy of the signed document if desired. The consent form was also signed and dated by the investigator (or his designee) and was retained as part of the study records.

2.2 Investigators and Study Administrative Structure

Principal Investigator:

Dr. Diego Moretti

Senior Researcher, ETH Zürich

Dr. Moretti was responsible and accountable for conducting the study. He assumes full responsibility for the treatment and evaluation of participants and for the integrity of the research data and results.

Other investigators:

Laura Hackl

Investigator/ Study Coordinator, ETH Zürich

Laura Hackl was primarily responsible for producing the investigational products, recruitment of the participants, administration of the test meals and the analysis of biological samples and study data.

Christophe Zeder, Adam Krzystek

Laboratory and Research Technicians ETH Zürich

Christophe Zeder was responsible for the stable isotope administrations and assessment as well as for the analytical quality control.

Adam Krzystek was responsible for the processing of preconditioned biological samples.

Prof. Dr. med Michael Zimmermann

Head of the Laboratory

Prof. Dr. med Zimmermann internally oversaw the conduction of the study and is the scientifically and administratively overseeing instance for this study.

2.3 Introduction

Iron deficiency is among the leading nutritional disorders in the world with high prevalence in vulnerable groups in both developing and industrialized countries (Kassebaum et al., 2014). It is considered the most common cause of anemia (Kassebaum et al., 2014), adversely affecting individuals on a physiologic level (Lozoff et al., 2014; WHO, 2001), which in consequence can also affect the economic performance (Horton and Ross, 2003; Plessow et al., 2015). Rice is a staple food for over 3 billion people worldwide (Muthayya et al., 2014), however, many nutrients in the rice are lost during the milling process (Doesthale et al., 1979). Given its high consumption rate in many deficient countries, rice is a logical vehicle for food fortification (Cook and Reusser, 1983).

Several technologies for rice fortification exist, whereas hot extrusion is recommended as it provides products with beneficial properties in terms of nutrient retention during rinsing, washing, cooking and shelf stability, cooking behaviour, visual appearance and cooked rice texture (Steiger et al., 2014). Feasibility and efficacy of rice fortification with iron have been shown in many studies [5, 12]. Several studies published in the literature that have shown efficacy of extruded fortified rice have employed cold or warm extruded rice formulations (Hotz et al., 2008; Moretti et al., 2006a), and one study investigating the efficacy of hot extruded rice did not find evidence for an increase in iron status (Thankachan et al., 2012).

It has been suggested that kernels produced with warm extrusion would be somewhat more resistant to digestion and do not easily release FePP (a water insoluble iron compound) in the duodenum. While the reasons for the findings in Thankachan et al study are most likely due to the repleted iron status of the study population, a notion supported by the improvement in vitamin B_{12} status, it cannot be completely ruled out that hot extrusuion may have a detrimental effect on iron bioavailability compared to cold or warm extrusion.

However, so far, research has mainly focussed on finding the most sensory acceptable compounds for fortification regarding sensory properties and bioavailability, and a direct comparison between hot and cold extruded rice has never been conducted. This study aimed to compare iron absorption from hot and cold extruded FePP fortified rice.

2.4 Study Objectives

The influence of extrusion conditions on the bioavailability of FePP from hot or cold extruded fortified rice was investigated. This study aimed to shed light on the question, whether differences exist in the iron absorption from FePP fortified rice extruded under hot or cold conditions.

2.5 Study Design

Single blind, randomized, cross-over design

2.6 Selection of Study Population

2.6.1 Inclusion criteria

- Female, 18 to 45 years old
- Normal body Mass Index (18.5 – 25 kg/m^2)
- Body weight < 67 kg
- Signed informed consent

2.6.2 Exclusion criteria

- Pregnancy (assessed by a pregnancy test), intention to become pregnant during the study or absent method of contraception
- Lactating/ Breastfeeding
- Any metabolic, gastrointestinal kidney or chronic disease such as diabetes, hepatitis, hypertension, cancer or cardiovascular diseases (according to the participants own statement)
- Continuous/long-term use of medication during the whole study (except for contraceptives)
- Consumption of mineral and vitamin supplements within 2 weeks prior to 1st meal administration and during the intervention time
- Blood transfusion, blood donation or significant blood loss (accident, surgery) over the past 4 months and during the intervention time
- Earlier participation in a study using Fe or Zn stable isotopes or participation in any clinical study within the last 30 days
- Participants who cannot be expected to comply with the study protocol (e.g. not available on certain study appointments)
- Smoking
- Vegan diet
- Extensive alcohol intake
- Drug abuse

2.6.3 Withdrawals

Two participants withdrew from the study before the first meal administration as they could not comply with the study protocol, the participants were replaced and their data were not included.

Other potential reasons for a withdrawal from the study are listed below.

- Incomplete consumption or missing of any test meal
- Incomplete or no blood collection at appointments designated for blood collection
- Consumption of food within 3 hours after test meal administration
- Vomiting within 3 hours after test meal administration
- Voluntary withdrawal of a participant
- For participants who needed to take any medication during the course of the study, withdrawal or postponement of the test meal were evaluated with respect to the used medication and its potential influence on iron absorption

For participants withdrawn from the study no more data were collected and no follow-up was conducted.

2.6.4 Procedures for the intervention including timeline

Procedure	Completion Date	Comments
Ethics Protocol approved (including amendment)	2015/07/23	
Production and analysis of labelled fortified rice	2015/07/30	
Screening of participants	2015/09/03	28 participants screened
Recruitment of study participants	2015/09/04	21 participants
Withdrawal from study procedure	2015/08/07	2 participants
Endpoint (last participant)	2015/10/02	
Sample analysis	2015/08/11	19 participants
Statistical analysis	2015/10/26	19 participants included

2.6.5 Investigational Product administered

The iron fortified rice samples fortified with ^{57}FePP, micronutrients and ZnO can be seen as the investigational products.

Test meal acronym/ fortification method	Description
^{57}FePP_H	Hot extruded rice containing ^{57}FePP, ZnO and a vitamin premix[1]
^{57}FePP_C	Cold extruded Rice containing ^{57}FePP, ZnO and a vitamin premix[1]

[1]Premix including vitamin A, thiamine, folic acid and vitamins B_1, B_3, B_6 and B_{12}

2.6.6 Investigational product(s)

Extruded Rice was produced by the Laboratory of Human Nutrition at ETH Zurich using a Brabender single-screw extruder (DSE 20/24 DO-Corder DN20; Brabender GmbH & Co KG, Duisburg, Germany) with a custom-made brass die and cutter under hot and cold extrusion conditions (**Table 1**). After extrusion, the extruded grains were air-dried overnight to reach a moisture content of approximately 10 %. Two different mixtures were prepared for extrusion **(Table 1).** Labelled ^{57}FePP was produced by Dr. Paul Lohmann GmbH KG (Germany), mimicking FePP powder (product nr. 505064001: d50: approx. 7 µm, d90: approx.

30 µm). Both mixtures were similar regarding ingredients and their quantities. The mixtures contained of rice flour, isotopically labelled FePP (^{57}FePP), ZnO, Citric Acid (CA) and a vitamin premix (vitamin A, thiamine, folic acid and vitamins B_1, B_3, B_6 and B_{12}). The mixtures only differed in their water content prior extrusion and in the extrusion temperature. The nutrient contents per serving are given in **Table 3).**

Table 1 - Extrusion conditions chosen for the production of extruded rice kernels.

Meal	Cold extrusion ^{57}FePP_C	Hot extrusion ^{57}FePP_H
Water amount (in g added prior extrusion respective to 100 g rice flour)	30.6	20.1
Temperature [°C][1,2]	45 – 48	80 – 81
Feeding rate [kg/h]	0.26 – 0.30	0.26 – 0.30
Pressure [Bar]	91 – 115	80 – 120
Drive [rounds/min]	120	120

[1]Measured with a temperature sensor in the extruder lumen (rice flour-water temperature).

[2]In cold extrusion the barrel temperature was actively cooled to a temperature of ≈40°C, while in hot extrusion the barrel temperature heated to ≈90°C.

2.6.7 Method of assigning subjects to intervention groups

The study had a randomized control design, each participant received both meals and acted as her own control during the study. The order of consumption was randomized individually. The randomization was single-blinded, therefore the participants did not know which type of test meal they were served at a given time point. The randomization list for the order of the meal consumption was generated in Microsoft Excel (Version Office 2010, Microsoft, Seattle).

2.6.8 Preparation of the meals

Two g extruded rice (exact weight was record) and 48 g regular Basmati rice were mixed in a heat-resistant glass bowl. One day prior to the administration, the unwashed rice was cooked in water for 20 minutes at 220°C at a 1:2 ratio (w:w) (Moretti et al., 2006b) and subsequently refrigerated at 5 °C. Before the study start, a vegetable sauce was prepared in bulk according to an earlier described procedure (Moretti et al., 2006b). The sauce was deep-frozen until one day prior to administration, when it thawed at 5 °C until the administration. On the administration day, 30 g of the vegetable sauce were added to the rice and the meal was heated in a microwave oven for 60 s at 600 W. This procedure was the same for both conditions.

2.6.9 Procedure for participants

All participants consumed their meals after an overnight fast. First, each participant was questioned by the investigator regarding medical issues and then underwent venipuncture conducted by a study nurse. Afterwards, the participant received her meal along with 300 ml Nanopure water. After complete consumption of the meal, the bowl was rinsed with a total amount of 20 ml Nanopure water, which had to be completely consumed by the participant to guarantee quantitative isotopic administration. The participants were asked to fast again for three hours after the meal consumption.

The same procedure was followed 14 days later, when the participants received their second test meal. An overview on the procedures is given below **(Figure 1).**

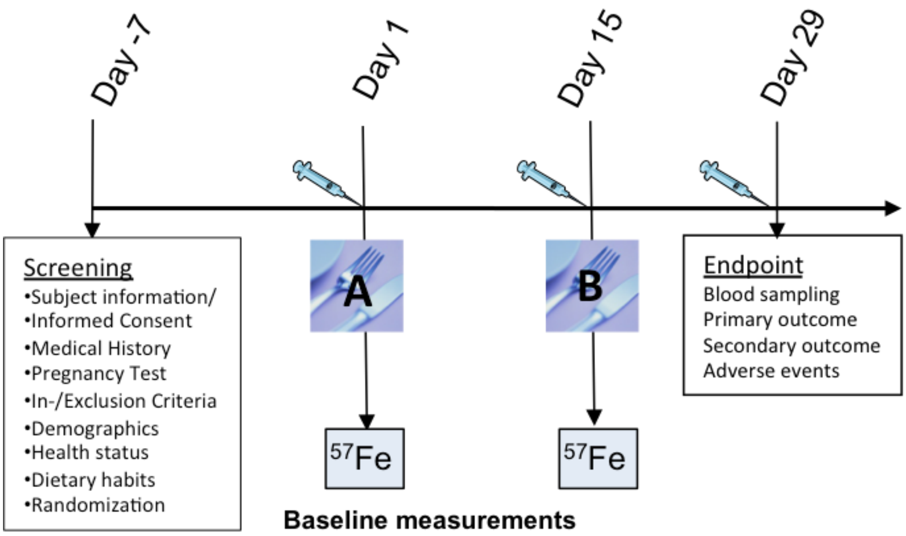

Figure 1. Study Flow Chart
Each participant consumed the isotopically labelled test meals A and B in a randomized order on days 1 and 15. Blood samples were taken on days 1, 15 and 29 (Endpoint).

2.6.10 Statistical and analytical procedures

The fractional iron absorption from the different meals within the same participant was compared by paired t-test. Differences were considered as significant at P <

0.05. All data were converted to their logarithms for statistical analysis, unless distributed normally, and reconverted for reporting.

Data processing and statistical analysis were performed with SPSS (version 22.0, 2013; SPSS Inc, Chicago, IL) and with Microsoft Excel (2013; Microsoft Corporation, Redmond, WA).

2.6.11 Determination of sample size

Sample size calculations indicated that with an SD of 0.28 (log transformed fractional iron absorption from a previous ETH study using FePP (Zimmermann et al., 2011) a type I error rate of 5% and 80% power the difference in absorption of 30% can be detected with a sample size of 16 participants. Because we anticipated some drop-outs during the 4 weeks study, and to ensure the study power, we recruited 20 participants (anticipated 20 % attrition).

2.7 Efficacy Evaluation

2.7.1 Efficacy Results

2.7.1.1 Participant's characteristics

Twenty participants were included in the study, whereas two participants dropped out before the first meal administration; one participant was replaced resulting in 19 participants included in the analysis. Age, anthropometric features, haemoglobin(Hb), plasma ferritin (PF) and c-reactive protein (CRP) concentrations at baseline (day of first meal administration) of those nineteen participants, who finished the study, are summarized below **(Table 2).**

Table 2- Table of anthropometric features iron and inflammatory status of the study participants assessed prior to first meal administration

Age[1], y	26.2 ± 3.4
Weight[1], kg	59.4 ± 4.8
Height[1], m	1.7 ± 0.1
BMI[1], kg/m^2	21.3 ± 1.6
Hemoglobin[1], g/l	134.7 ± 9.7

C-reactive protein[2], mg/l	0.8 (1.2)
Plasma ferritin[1], ng/ml	46.2 ± 30.5

Values are means[1] ± SDs or geometric means[2] (interquartile range), n = 19.

2.7.1.2 Test meal composition

The mean (±SD) native iron concentration of the unfortified Basmati Rice (Coop Qualité & Prix) was 3.8 µg/g (0.3), the iron concentration of the vegetable sauce was 30 µg/g dry matter. The phytate concentrations for the unfortified Basmati Rice were 0.14 g/100 g and for the vegetable sauce 0.01 g/100 g dry matter. Iron and phytate concentrations in the rice flour used for the extrusion were 3 µg/g and 0.18 g/100 g dry matter, respectively. The estimated values are based on values obtained from preliminary studies using similar resources (same products, but different batches). The extruded rice was mixed with the Basmati Rice at a 24 : 1 ratio to result in the desired fortification level of 4 mg iron per serving. This ratio was established in an earlier study in order to keep the unavoidable losses of labelled ferric pyrophosphate during the extrusion process as small as possible.

Table 3 - Test Meal Composition

	Nutrient amounts administered per serving of fortified rice	
Meal	**Cold extruded** **^{57}FePP_C**	**Hot extruded** **^{57}FePP_H**
Ratio Basmati : Extruded Rice [w/w]	24 : 1	24 : 1
Total iron content per meal [mg]	4.343 ± 0.003	4.436 ± 0.003
Total Zinc content per meal [mg]	4.027 ± 0.034	3.979 ± 0.040
Iron : Zinc Molar Ratio	1 : 0.8	1 : 0.9
Estimated Phytic Acid : Iron Molar Ratio*	1.60 : 1	1.60 : 1
Fractional Iron Absorption [%]	2.99[a]	1.50[b]

Total absorbed iron per meal [mg]	0.135[a]	0.150[b]
Vitamin Premix	Vitamin A 366,3 IU, Folic Acid 0,15 mg, Niacin 4 mg, Vitain B_1 0,4 mg, Vitamin B_6 0,4 mg, Vitamin B_{12} 1,1 µg	

Values are geometric means with a 95 % CI. Different superscript letters in a row indicate significant differences between values at $P < 0.05$
^{57}FePP_C: Extruded rice containing ^{57}FePP, ZnO, CA and vitamin premix , produced under cold extrusion conditions. ^{57}FePP_H: Extruded rice containing ^{57}FePP, ZnO, CA and vitamin premix, produced under hot extrusion conditions.
*Estimated values are based on preliminary studies using comparable resources.

2.7.1.3 Preparation of the meals

The unwashed rice was cooked in water for 20 minutes at 220°C at a 1:2 ratio (w:w) (Moretti et al., 2006b) and subsequently refrigerated at 5 °C until the time of administration. Prior to consumption, 30 g vegetable sauce were added and the meals were heated in a microwave oven for 60 s at 900 W.

2.7.1.4 Iron absorption

Fractional iron absorption from meal FePP_C differed significantly from meal FePP_H ($P < 0.05$). The absorption was almost double in the cold extruded rice compared to the hot extruded one.

Our findings show that the extrusion condition has an influence on iron absorption and that iron bioavailability from cold extruded rice can be higher than from hot extruded rice.

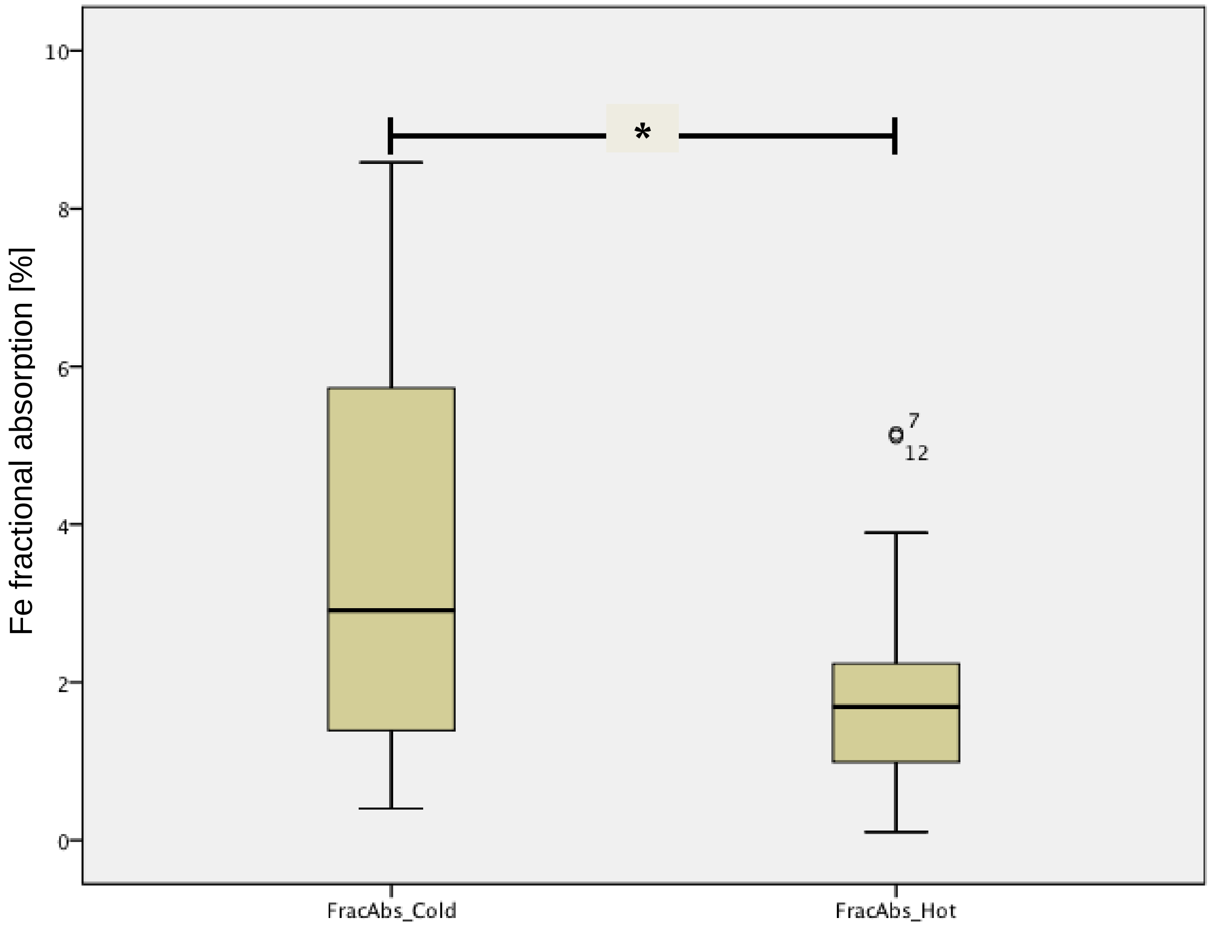

Figure 2. Boxplots for fractional iron absorption [%] from two different meals. The horizontal bar shows the median fractional absorption for each meal. Each box represents the first to third quartile, the whiskers represent the lowest and highest data points. Circles indicate outliers; Asteriks show significant differences between the different meals.

3 DETERMINATION OF IN VITRO IRON SOLUBILITY AND SENSORY PROPERTIES OF EXTRUDED FORTIFIED RICE

3.1 Determination of in vitro solubility

We developed a rapid assay for determining iron solubility from rice in vitro based on an earlier described method (Miller et al., 1981). Therewith, we were able to screen different iron-fortified extruded rice kernels for their potential in iron bioavailability with higher time and resource efficiency. In vitro trials were based on the results reported in 1.1, indicating a doubling of iron absorption when a citric acid/ trisodiumcitrate (CA/TSC) mixture was co-extruded with FePP in iron fortified rice. Here we investigate in detail which quantity and ratio delivers the highest in vitro solubility for further optimization.

3.1.1 Preparation of fortified extruded rice grains for *in vitro* trials

Different iron compounds were screened for iron solubility. The desired iron content in all screened compounds was always 5 mg/g, unless indicated differently.

The preparation of the rice grains produced for the *in vitro* experiments was similar to the one for isotopically labeled hot extruded rice; the only difference was that either regular FePP (124466; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany) or $FeSO_4$ (Ferrous sulfate-7-hydrate 20% iron; 288096; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany) was used and no vitamins were added. Thus, the fortified extruded rice grains contained rice flour, FePP or $FeSO_4$ and either CA/TSC (in varying molar ratios relative to FePP) or no CA/TSC. One type of rice grains contained SFP with sodium citrate (SFP + SC, 30109381; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany). Additionally, some samples were extruded with ZnO (D99013; Jungbunzlauer Suisse AG, Basel, Switzerland).

Iron content of the rice samples was determined by atomic absorption spectrophotometry (AAS) prior to in vitro testing.

3.1.2 In vitro solubility trial for iron in rice

All extruded samples were prepared in triplicate or duplicate, for some samples, unfortified long-grain rice (Jasmine rice Bio, Migros, Switzerland) with a $FeSO_4$ solution served as a reference. Thirty ml boiling water were added to 1 g extruded or unfortified rice, respectively, and cooked in an oven (BOSCH, Switzerland) for 20 minutes at 150°C. After cooling down to 35-40 °C and homogenization with a Polytron homogenizer (PT 1200 E; Kinematica AG, Lucerne, Switzerland), aliquots of 250 mg were taken in triplicate from each sample for mineralization and subsequent determination of total iron concentration. 1 ml of a $FeSO_4$ solution (5 mg/ml) was added to the unfortified reference sample before aliquoting.
To the remainder of each sample, 0.1 g amylase (Takadiastase from *Aspergillus oryzae*, BCBM 5345; Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) were added and samples were incubated for 10 minutes at room temperature with subsequent adjustment to pH 2 with 6 M HCl. From each sample, three aliquots of 3 ml were mixed with 26 µl pepsin solution [1.6 g pep(ZnO, D99013; Jungbunzlauer Suisse AG, Basel, Switzerland), sin (Porcine, P70000; Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) in 10 g 0.1 M HCl] and were incubated on a shaking water bath for 2 h at 37 °C and 150 rpm. Thereafter, all

samples were centrifuged for 15 minutes at 3600 rpm and the iron concentration in the respective supernatants was measured. The values for each triplicate were summed up and this sum was extrapolated to the weight of the sample after initial cooking. The solubility [%] was expressed as the quotient of the prior extrapolated iron content measured in the supernatant divided by the total iron content in the respective aliquots.
An overview on the extruded rice samples, which were investigated for in vitro solubility, is given below **(Table 4).**

Table 4 Assessment of iron content and in vitro iron solubility

Extruded Rice	**Mean Fe content [mg/g] (SD)**	**Fractional iron solubility [%]**
FePP (1 : 0 : 0)	4.38 (0.14)	2.70[2]
FePP + CA + TSC (1 :0.09 : 1.69)	4.92 (0.17)	9.60[2]
FePP extruded + CA/TSC solution added before cooking (1 :0.09 : 1.69)	4.38 (0.14)	7.05[2]
FePP extruded + CA/TSC solution added after cooking (1 :0.09 : 1.69)	4.38 (0.14)	6.19[2]
SFP (+ sodium citrate) 1 : 3 : 2	2.56 (0.26)	8.10[2]
FePP + CA + TSC (1 :0.11 : 2.06)	4.23(0.29)	10.12[2]
FePP + CA + TSC (1 :0.13 : 2.40)	4.82 (0.22)	11.00[2]
FePP + CA + TSC (1 :0.25 : 4.70)	-	14.61[2]
FePP + CA + TSC (1 :0.92 : 17.17)	-	19.94[2]
$FeSO_4$ (1 : 0 : 0)	6.23 (0.14)	17.14[2]
$FeSO_4$ + CA + TSC (1 :0.09 : 1.69)	6.24 (0.02)	29.43[3]
$FeSO_4$ extruded + CA/TSC solution added before cooking (1 :0.09 : 1.69)	6.23 (0.14)	20.43[3]
$FeSO_4$ extruded + CA/TSC solution added after cooking (1 :0.09 : 1.69)	6.23 (0.14)	15.96[3]

Fractional iron solubility [%] for extruded rice samples respective to each samples total iron content. Iron content in each sample was 5 mg/g – Molar ratios of Fe : CA : TSC are given in brackets. [2] N = 3; [3] N = 2; CA = Citric Acid; FePP = Ferric Pyrophosphate, $FeSO_4$ = Ferrous Sulfate; TSC = Trisodium Citrate; SFP = Soluble Ferric Pyrophosphate

Table 5 Assessment of iron content and in vitro iron solubility

Extruded Rice	Mean Fe content [mg/g] (SD)	Mean Zn content [mg/g] (SD)	Fractional iron solubility [%]
FePP + CA + TSC + ZnO (1 :0.09 : 1.69 : 0.84)	5.27 (0.06)	4.84 (0.00)	8.82[2]
FePP + CA + TSC + ZnO (1 :0.09 : 1.69 : 1.68)	4.39 (0.06)	9.00 (0.61)	9.96[2]
SFP (+ sodium citrate) + ZnO (1 : 3 : 2 : 0.84)	4.64 (0.26)	4.54 (0.02)	7.00[2]
SFP (+ sodium citrate) + ZnO (1 : 3 : 2 : 1.68)	5.07 (0.09)	8.96 (0.28)	7.15[2]

Fractional iron solubility [%] for extruded rice samples respective to each samples total iron content. Iron content in each sample was 5 mg/g – Molar ratios of Fe : CA : TSC : Zn are given in brackets. [2] N = 2; CA = Citric Acid; FePP = Ferric Pyrophosphate, $FeSO_4$ = Ferrous Sulfate; TSC = Trisodium Citrate; SFP = Soluble Ferric Pyrophosphate; ZnO = Zinc Oxide

3.2 Determination of sensory properties of iron fortified rice

Visual appearance is a determining factors when it comes to acceptability of rice in consumers. Therefore, we screened several extruded rice samples with different iron compounds regarding their colour.

3.2.1 Preparation of fortified extruded rice grains for *in vitro* trials

The preparation of the rice grains produced for analysing their sensory properties was similar to the one for isotopically labelled hot extruded rice; the only difference was that either regular FePP (124466), $FeSO_4$ (288096), SFP + SC (30109381), Ferrous succinate (34 % Fe II; 1070205; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany), Ferrous ammonium phosphate (26.6% Fe II and 5.9% Fe III; 11082904; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany), Ferric sodium edate (13.7% Fe III; 10059043; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany), Ferric tartrate-1-hydrate (18.9% Fe III; 1022028; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany), Iron taste free SF (13211203; 21.06% Fe II;ALBION Human Nutrition Laboratories Inc., Clearfield, USA) or Ferrous bisglycinate (1050293-22; 2.8% Fe II; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany) were used and no vitamins were added. Thus, the fortified extruded rice grains contained rice flour, the respective iron compound and either CA/TSC (in varying molar ratios relative to FePP) or no CA/TSC.

Additionally, some samples were extruded with ZnO (D99013), Zinc sulfate ($ZnSO_4$, 1082451; 10.70% Zn in zinc sulfate-1-hydrate; Dr. Paul Lohmann GmbH KG, Emmerthal, Germany) or Titanium Dioxide (TiO_2; 59.95% TiO2 in titan(IV)-oxide (Merck KGaA, Darmstadt, Germany Charge Nr. 1115620). Iron content of the rice samples was determined by AAS prior to in vitro testing.

3.2.2 Visual properties of fortified rice samples

Visual properties of the rice samples were determined in triplicate with a Minolta Chromameter CR-310 (Minolta Camera Co., Osaka, Japan) as previously described (Pinkaew et al., 2012a). The color of the rice samples was expressed as ΔE values and was measured in triplicate on days 0, 1, 14, 28, 42, 56, 70, 84 to monitor colour changes over time. This can be an indicator for storage stability. All ΔE values were calculated relative to Basmati rice (Qualite & Prix, Coop, Switzerland). Day 0 indicates ΔE values at the day of extrusion, values for day 1 were measured after ~24 hours drying under ambient conditions.

Most of the rice samples showed a decrease in ΔE after day 1 with again an increase after day 14 **(Figure 3).** This pattern was not shown in samples *Iron taste free SF* and *Ferric tartrate 1-hydrate,* both of which started at relatively high ΔE in the beginning and were then stable throughout the whole measurement period. Of all presented iron compounds in Figure 3, FePP showed the lowest ΔE from days 14 to 84, which again confirms that it should be the compound of choice for iron fortification of rice.

Our in vitro investigations show a higher iron solubility with increasing CA : TSC molar ratios respective to iron. Extruded rice with a molar ratio of Fe : CA : TSC of 1 : 0.92 : 17.17 showed a fractional iron solubility (FAFeS) of 19.94 %, which was slightly higher than from extruded $FeSO_4$. These in vitro results show, that iron solubility from FePP can even surpass the one of $FeSO_4$ alone, when extruded with CA/TSC. Iron solubility from $FeSO_4$ could also be enhanced to 29.43 % (FAFeS) when adding CA/TSC at extrusion at a molar ratio of Fe (as SO_4) : CA : TSC 1 :0.09 : 1.69, whereas it was only slightly higher, when CA/TSC was added as a solution after extrusion (same molar ratio, FAFeS = 20.43 %). Nevertheless, due to its interaction with the rice matrix $FeSO_4$ should only be used in special cases (Steiger et al., 2014).

The extruded compounds containing FePP + CA + TSC all showed similar ΔE values, ΔE increased with increasing CA/TSC addition. Whereas FePP + CA + TSC (1 : 0.11 : 2.03) did not follow this pattern **(Figure 4).** Rice extruded with CA + TSC only, without FePP, showed the lowest ΔE after 28 days with relatively consistent values from days 42 to 70 and a further decrease between days 70 and 84. The commercially available Soluble ferric pyrophosphate exhibited the highest ΔE values.

An evaluation of the different color masking compounds **(Figure 5),** exhibited the lowest ΔE values for FePP + CA + TSC + TiO_2. In general, all ΔE values were lower, when any of the masking compounds ($ZnSO_4$, ZnO, TiO_2) was added, however, when evaluating the images of the extruded rice samples, it is clear that TiO_2 lead to an unnatural off-color **(Figure 6).** This off-coloration might improve when lowering the amounts of added TiO_2. While the use TiO_2 has been suggested to be safe in small amounts, it can have non-toxic effects at higher doses (Koeneman et al., 2010).

Zinc compounds, on the other hand, have the advantage, that they have been suggested for rice fortification (Pee, 2014). In terms of color, both zinc compounds exhibited satisfying results **(Figure 8),** whereas $ZnSO_4$ seems slightly advantageous when added to FePP : CA : TSC at molar ratios of 1 : 0.09 : 1.69. In a recent human study, we showed that $ZnSO_4$ does not impair iron absorption from FePP and zinc fortified rice, whereas ZnO does (unpublished results). The addition of $ZnSO_4$ to rice containing retinyl palmitate (RP) and micronized ground

FePP (MGFP) has shown improved color stability, however, RP retention was significantly lower after 18 weeks compared to rice containing ZnO, RP and MGFP. In terms of RP stability, color and price, it has therefore been suggested, to use ZnO in triple fortified rice (Pinkaew et al., 2012a).

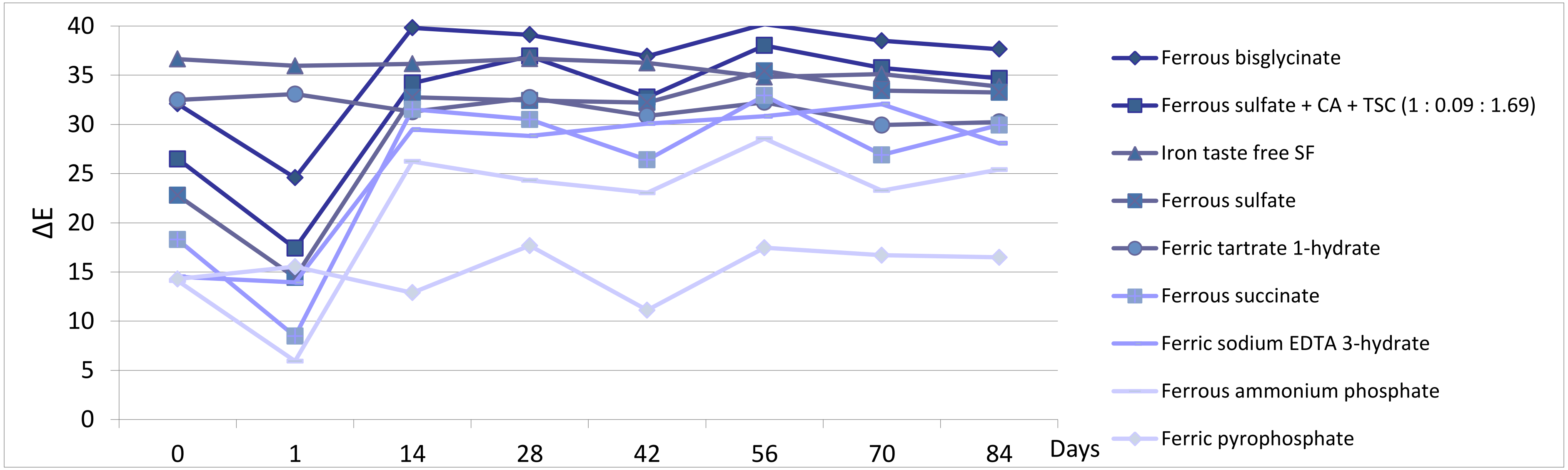

Figure 3. ΔE measurements for extruded rice samples fortified with different iron compounds (5 mg/g iron) compared to Basmati rice over 84 days.

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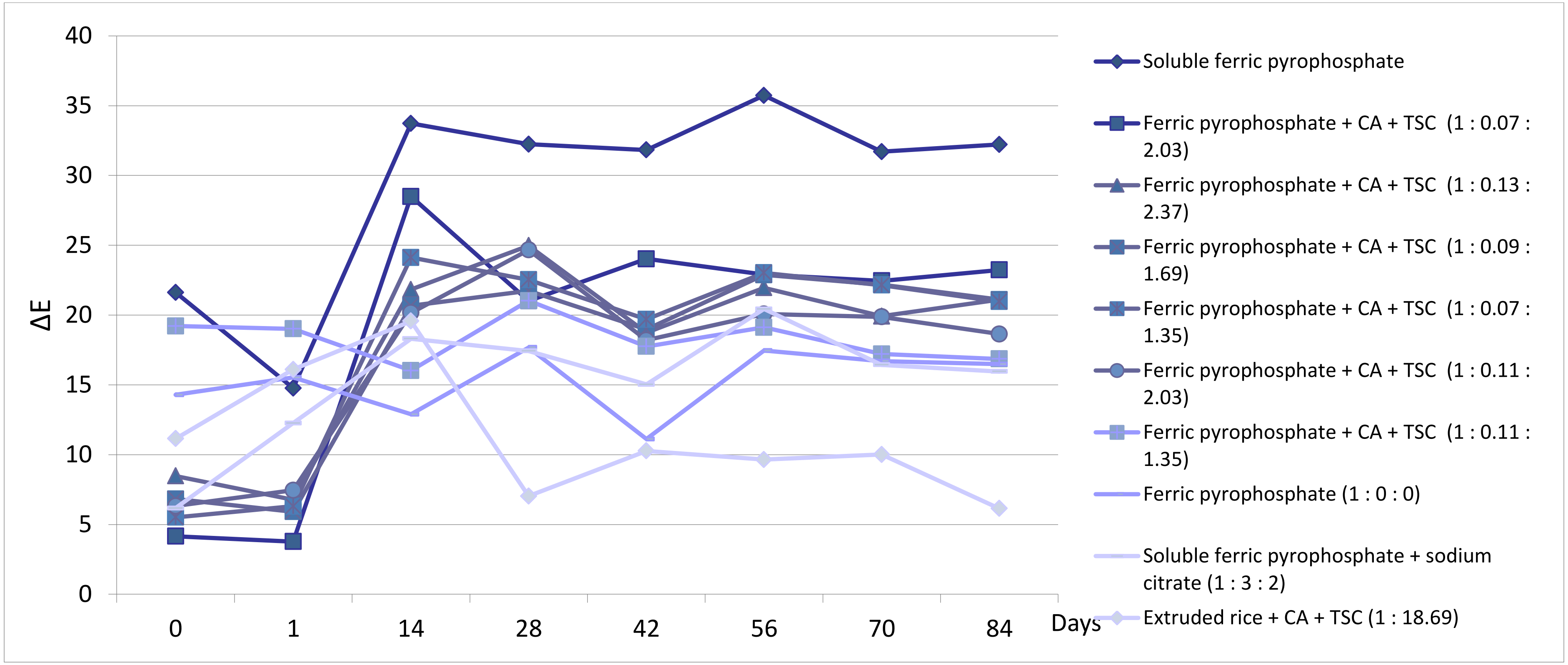

Figure 4. ΔE measurements for extruded rice samples fortified with varying Fe : CA : TSC (5 mg/g iron) molar ratios and for SFP-fortified rice compared to Basmati rice over 84 days.

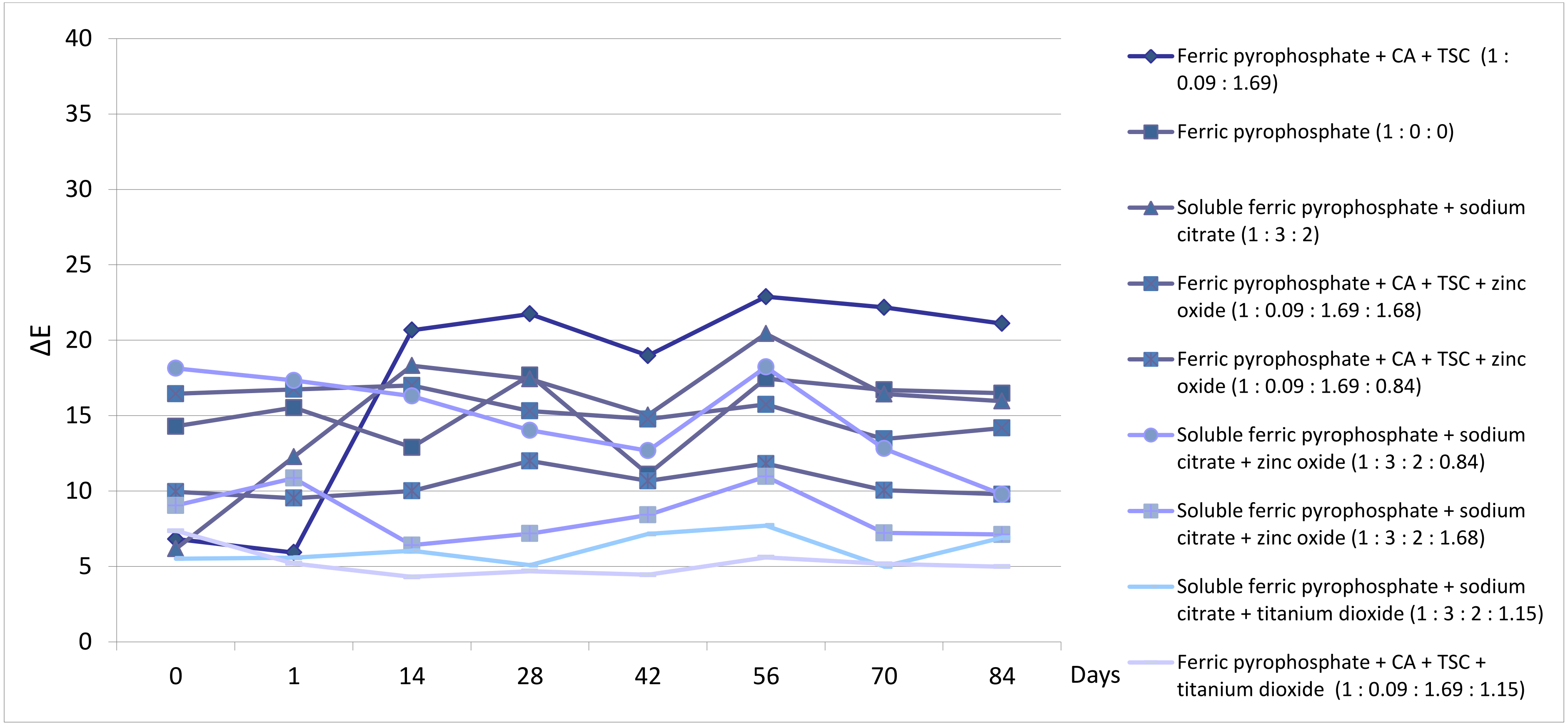

Figure 5. ΔE measurements for extruded rice samples fortified with varying FePP (5 mg/g iron), CA, TSC either ZnO or TiO2 as color masking agents. Samples were compared to Basmati rice over 84 days.

Compound	Rice sample	+ CA/TSC (1.58/45.20 mg/g)	+ZnO (5 mg/g)	+ZnO (10 mg/g)	+ TiO_2 (5 mg/g)

Unfortified extruded rice

Ferric pyro-phosphate (5 mg/g iron)

Soluble ferric pyrophosphate (5 mg/g iron) **with sodium citrate**

Figure 6. Unfortified extruded rice, CA/TSC addition alone and FePP extruded rice samples including CA/TSC (1 : 0.09 : 1.69) and SFP + SC (1 : 3 :2) with selected color masking compounds.

Compound	+ CA/TSC (0.07 : 1.38)	+ CA/TSC (0.09 : 1.69)	+ CA/TSC (0.11 : 2.03)	+ CA/TSC (0.13 : 2.37)	+ CA/TSC (0.20 : 3.79)

Ferric pyro-phosphate (5 mg/g iron)

Figure 7. FePP fortified rice with varying CA/TSC molar ratios.

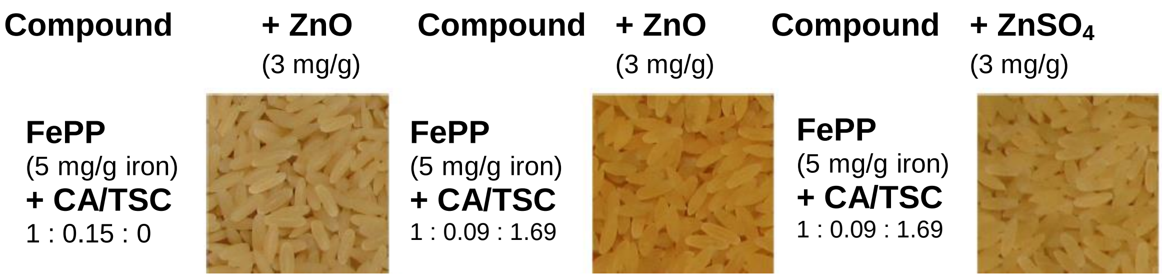

Figure 8. FePP and CA/TSC extruded rice with different Zinc compounds.

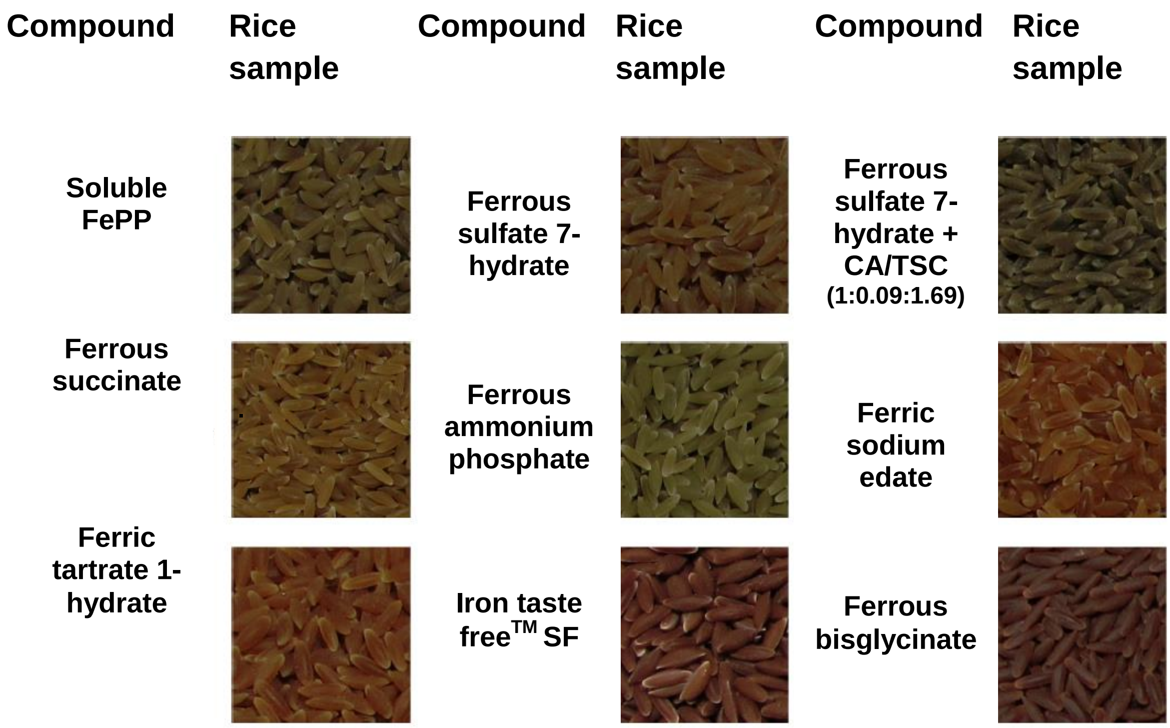

Figure 9. Extruded iron fortified rice with different iron compounds (5 mg/g iron).

4 CONCLUSIONS

Our findings from the human study show that using cold extruded iron fortified rice leads to higher iron absorption from FePP than does hot extruded rice. Physicochemical properties of extrudates depend on the extrusion temperature (Zhuang et al., 2010) – it is therefore not surprising, that iron absorption differs from rice extruded at different temperatures. The composition of the rice mixtures differed only in water content and extrusion temperature. However, the water content in both rice samples was most likely equilibrated around 10 % after extrusion and overnight drying. Cold extruded rice has lower stability during cooking and it is likely that the looser structure of the grain facilitated iron bioavailability from FePP. As a water insoluble iron compound, FePP requires diluted acid for quantitative dissolution and absorption by the duodenal enterocytes and it is possible that the tighter structure of the hot extruded kernels negatively affects this dissolution, which has to take place in relatively short time after stomach-passage as iron absorption is limited to the duodenum.

One limitation of this study is that no reference meal was administered and we were therefore not able to calculate the relative bioavailability for FePP from the two test meals. Another limitation is that the hot extruded batch was produced 2 months before the cold extruded one. However, it has been suggested, that ageing has no influence on starch retrogradation of rice flour, when kept at room temperature (Teo et al., 2000).

We recently showed, that FePP + CA + TSC extruded rice significantly enhances human iron absorption compared to FePP fortified rice, where CA/TSC is added as equimolar solution. An iron solubility cutoff with increasing ratios of CA/TSC : FePP in extruded fortified rice is yet to be found, however, increasing amounts of CA/TSC in FePP extruded rice lead to further discoloration, which leads to sensory inacceptable rice kernels. This can only partly be compensated by the addition of zinc as color masking agent. Nevertheless, it should be investigated in more detail, what the threshold for iron solubility from rice extruded with FePP and CA/TSC is.

After thorough screening of different iron compounds, FePP still remains the only compound that we conclude as being useable for rice fortification. However, ferric ortho-phosphate might be a promising compound for rice fortification and should be investigated regarding its iron solubility and possibly its bioavailability in rice, as it has been reported to be less costly than FePP and to have better sensory properties (whiter colour). Early studies from a range of foods suggest ferric orthophosphate to have ≈50% bioavailability than $FeSO_4$ (Hallberg et al., 1989)

Whether cold extrusion should be preferred over hot extrusion is yet to be investigated, especially with respect to storage stability and vitamin retention. The addition of CA/TSC to FePP fortified rice is highly recommended, however, the fortification level has to be chosen carefully to ensure consumers' acceptability as the addition of CA/TSC has a detectable effect on the color of the extruded grains.

In terms of color masking, we suggest to use zinc compounds, as zinc is a nutrient that should be within a fortified food. Further investigations on storage stability and matrix-interactions should be conducted before drawing a clear conclusion as to which zinc compound is the compound of choice for rice fortification.

To confirm programmatic relevance of these findings it is suggested to implement a study investigating fortified rice within a target population (iron deficient anemic) over an extended time period at currently used rice kernel premix : natural rice ratios (blending ratio of 1 : 100).

4 PROGRAMMATIC IMPLICATIONS

These results highlight the peculiar characteristics of iron fortified rice which depend both on the fact that rice is fortified with a premix kernel approach and on the use of FePP as the iron fortification compound. While FePP in rice has been shown to be efficacious in improving the iron status in iron deficient populations, its bioavailability remains low and better absorption would allow to reduce fortification levels, decreasing costs and possibly required kernel premix blending ratios.

Our results strongly suggest the use of a chelating substance for the enhancement of iron bioavailability from rice, such as CA/TSC. Further chelating substances such as Natrium pyrophosphate or NaEDTA (Johns et al., 2015) may also be used, but have yet to be investigated in rice in vivo. Nonetheless, our results are only based on one single human study conducted in iron replete female subjects, using a single meal approach. Further research in at risk populations (affected by iron deficiency and anaemia) and using a multiple meal design with employing fortification levels consistent with a hypothetic program appear warranted.

As the use of chelating substances in rice fortification with FePP may become the norm, the negative effect of ZnO co-fortification on rice bioavailability needs to be evaluated in presence of these substances. However if confirmed, our results suggest that rice fortification with Zinc may be more advantageous when performed with $ZnSO_4$. A downside of this approach would be the lower reported stability of Vitamin A in fortified rice when $ZnSO_4$ is used as fortificant (Pinkaew et al., 2012b). Such an option should therefore be envisaged only if other vehicles for vitamin A fortification are available such as fortification of vegetable oils.

Our results also point out that cold extrusion may offer a better FePP bioavailability over hot extrusion. While cold extrusion is typically applied in the food industry in pasta production processes, the cooking stability of extruded rice kernels after cooking is not comparable to hot extruded kernels. However, our results suggest a better bioavailability of cold extruded rice.

FePP and Ferric orthophosphate currently appear to be the only suitable iron compounds in iron fortified rice with acceptable sensory profiles.

5 REFERENCES

Cook, J. D., and Reusser, M. E., 1983, Iron fortification: an update: The American journal of clinical nutrition, v. 38, no. 4, p. 648-659.

Doesthale, Y. G., Devara, S., Rao, S., and Belavady, B., 1979, Effect of milling on mineral and trace element composition of raw and parboiled rice: Journal of the Science of Food and Agriculture, v. 30, no. 1, p. 40-46.

Hallberg, L., Rossanderhulthen, L., and Gramatkovski, E., 1989, Iron Fortification of Flour with a Complex Ferric Ortho-Phosphate: American Journal of Clinical Nutrition, v. 50, no. 1, p. 129-135.

Horton, S., and Ross, J., 2003, The economics of iron deficiency: Food Policy, v. 28, no. 1, p. 51-75.

Hotz, C., Porcayo, M., Onofre, G., Garcia-Guerra, A., Elliott, T., Jankowski, S., and Greiner, T., 2008, Efficacy of iron-fortified Ultra Rice in improving the iron status of women in Mexico: Food and Nutrition Bulletin, v. 29, no. 2, p. 140-149.

Johns, P. W., Patel, G. C., Parker, M. E., Lasekan, J. B., Milani, P., Nixon, M. K., Tigner, M., and Schmitz, D. J., 2015, Evaluation of the effect of Ultra Rice((R)) EDTA supplementation on the soluble iron, visual acceptance and vitamin A stability of commercial milled rice blends: International Journal of Food Science and Technology, v. 50, no. 7, p. 1615-1624.

Kassebaum, N. J., Jasrasaria, R., Naghavi, M., Wulf, S. K., Johns, N., Lozano, R., Regan, M., Weatherall, D., Chou, D. P., and Eisele, T. P., 2014, A systematic analysis of global anemia burden from 1990 to 2010: Blood, v. 123, no. 5, p. 615-624.

Koeneman, B., Zhang, Y., Westerhoff, P., Chen, Y., Crittenden, J., and Capco, D., 2010, Toxicity and cellular responses of intestinal cells exposed to titanium dioxide: Cell Biology and Toxicology, v. 26, no. 3, p. 225-238.

Lozoff, B., Castillo, M., Clark, K. M., Smith, J. B., and Sturza, J., 2014, Iron Supplementation in Infancy Contributes to More Adaptive Behavior at 10 Years of Age: The Journal of nutrition, v. 144, no. 6, p. 838-845.

Miller, D. D., Schricker, B. R., Rasmussen, R. R., and Van Campen, D., 1981, An in vitro method for estimation of iron availability from meals: The American Journal of Clinical Nutrition, v. 34, no. 10, p. 2248-2256.

Moretti, D., Zimmermann, M. B., Muthayya, S., Thankachan, P., Lee, T. C., Kurpad, A. V., and Hurrell, R. F., 2006a, Extruded rice fortified with micronized ground ferric pyrophosphate reduces iron deficiency in Indian schoolchildren: a double-blind randomized controlled trial: American Journal of Clinical Nutrition, v. 84, no. 4, p. 822-829.

Moretti, D., Zimmermann, M. B., Wegmüller, R., Walczyk, T., Zeder, C., and Hurrell, R. F., 2006b, Iron status and food matrix strongly affect the relative bioavailability of ferric pyrophosphate in humans: The American journal of clinical nutrition, v. 83, no. 3, p. 632-638.

Muthayya, S., Sugimoto, J. D., Montgomery, S., and Maberly, G. F., 2014, An overview of global rice production, supply, trade, and consumption: Annals of the New York Academy of Sciences, v. 1324, no. 1, p. 7-14.

Pee, S., 2014, Proposing nutrients and nutrient levels for rice fortification: Annals of the New York Academy of Sciences, v. 1324, no. 1, p. 55-66.

Pinkaew, S., Wegmuller, R., and Hurrell, R., 2012a, Vitamin A stability in triple fortified extruded, artificial rice grains containing iron, zinc and vitamin A: International Journal of Food Science & Technology, v. 47, no. 10, p. 2212-2220.

-, 2012b, Vitamin A stability in triple fortified extruded, artificial rice grains containing iron, zinc and vitamin A: International Journal of Food Science and Technology, v. 47, no. 10, p. 2212-2220.

Plessow, R., Arora, N. K., Brunner, B., Tzogiou, C., Eichler, K., Brügger, U., and Wieser, S., 2015, Social Costs of Iron Deficiency Anemia in 6–59-Month-Old Children in India: PLoS ONE, v. 10, no. 8, p. e0136581.

Steiger, G., Muller-Fischer, N., Cori, H., and Conde-Petit, B., 2014, Fortification of rice: technologies and nutrients: Technical Considerations for Rice Fortification in Public Health, v. 1324, p. 29-39.

Teo, C., Karim, A. A., Cheah, P., Norziah, M., and Seow, C., 2000, On the roles of protein and starch in the aging of non-waxy rice flour: Food Chemistry, v. 69, no. 3, p. 229-236.

Thankachan, P., Rah, J. H., Thomas, T., Selvam, S., Amalrajan, V., Srinivasan, K., Steiger, G., and Kurpad, A. V., 2012, Multiple Micronutrient-Fortified Rice Affects Physical Performance and Plasma Vitamin B-12 and Homocysteine Concentrations of Indian School Children: Journal of Nutrition, v. 142, no. 5, p. 846-852.

WHO, 2001, Iron Deficiency Anemia: World Health Organization.

Zhuang, H., An, H., Chen, H., Xie, Z., Zhao, J., Xu, X., and Jin, Z., 2010, EFFECT OF EXTRUSION PARAMETERS ON PHYSICOCHEMICAL PROPERTIES OF HYBRID INDICA RICE (TYPE 9718) EXTRUDATES: Journal of Food Processing and Preservation, v. 34, no. 6, p. 1080-1102.

Zimmermann, M. B., Biebinger, R., Egli, I., Zeder, C., and Hurrell, R. F., 2011, Iron deficiency up-regulates iron absorption from ferrous sulphate but not ferric pyrophosphate and consequently food fortification with ferrous sulphate has relatively greater efficacy in iron-deficient individuals: British Journal of Nutrition, v. 105, no. 08, p. 1245-1250.