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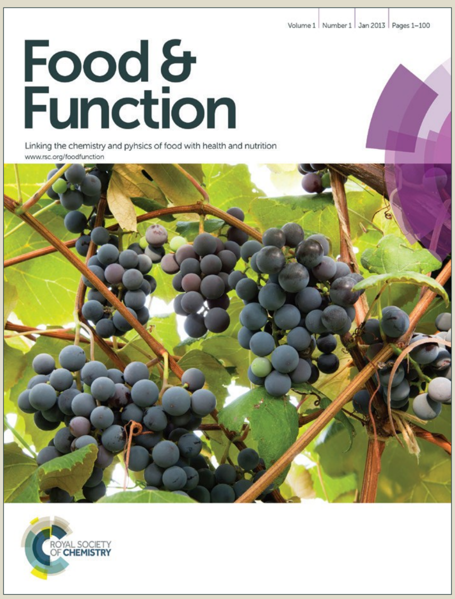

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The Type of Fortificant and the Leaf Matrix Both Influence Iron and Zinc Bioaccessibility in Iron-fortified Green Leafy Vegetable Sauces from Burkina Faso

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Leafy vegetable sauces from Burkina Faso were assessed as a potential vehicle for food fortification. First, iron and zinc bioaccessibility were measured by dialysability method in amaranth and Jew's mallow sauces and in traditional whole dishes consisting of maize paste plus leafy vegetable sauces. Iron dialysability and solubility were higher in amaranth than in Jew's mallow sauce, pointing to a marked effect of the matrix. Iron dialysability was hardly affected by the maize paste contrary to zinc dialysability, which was reduced. Second, iron and zinc bioaccessibility was assessed in the same sauces fortified with NaFeEDTA or iron sulfate. Added iron, i.e. iron supplied by fortification, represented 60% of total iron at the low fortification level and 80% at high level. In amaranth sauces with the high level of fortification using NaFeEDTA and iron sulfate, fractional dialysable iron reached respectively 66% and 26% compared to only 8.1% in the unfortified sauce. Similarly, in Jew's mallow sauces, fractional dialysable iron was 57% and 5% respectively with NaFeEDTA and iron sulfate and less than 1% in the unfortified sauce. Concomitantly, fractional dialysable zinc increased by respectively 20% and 40% in amaranth and Jew's mallow sauces fortified with NaFeEDTA whereas it remained unchanged with iron sulfate. Iron fortification could be an efficient way to greatly increase the available iron content of green leafy vegetable sauces and for this purpose NaFeEDTA is more effective than iron sulfate whatever the food matrix.

1 Introduction

Traditional diets in African low-income countries rely on starchy foods prepared from staple crops, accompanied by a relish, sauce or soup, consisting of vegetables and often leafy vegetables, beans or groundnuts, fats and oils, condiments and spices. [1] Leafy vegetables are frequently consumed, almost daily, mainly as sauces in Western Africa, as they make starchy foods more palatable[2] and are a well-known source of micronutrients. [3-5] They could thereby be used in food-to-food fortification programs to help fight widespread micronutrient deficiencies in low-income countries. [6-7]
In Burkina Faso in 2010, anemia prevalence was 88% in children aged 6-59 months, and almost 49% in women aged 15-49 years. [8] Anemia can be caused by several factors like parasitic infections, bleeding, chronic diseases… and about half cases are due to iron deficiency. [9] Data about zinc deficiency are scarce and not available in Burkina Faso. However, it is well recognized that growth retardation is one of its main consequences, and its national prevalence in Burkina Faso is 34.1%. [8] Leafy vegetables could thus supply iron and are a good, available, cheap and natural source of vitamin C and provitamin A. As outlined by some authors[10], leafy vegetables could play a role in anemia prevention thanks not only to the presence of iron but also to their significant ascorbic acid content, which is known to promote the absorption of soluble non-haem iron by chelating the iron thus maintaining it in the ferrous absorbable soluble form. [11] Despite consumption patterns that differ among households in different countries[10], this natural resource, which is harvested in the wild[12] or cultivated, can contribute to food and nutrition security. [13,6] The consumption of leafy vegetables should thus be encouraged. Food processing may affect their nutritional value: cooking has no effect on iron and zinc content but reduces ascorbic acid content and may thus affect the bioavailability of these micronutrients; it also increases beta-carotene bioavailability by softening the plant structure. [10]
Few studies have assessed the bioavailability of iron and zinc in leafy vegetable dishes. A higher *in vitro* available iron fraction was measured in leafy vegetables from India or leafy vegetable-based meals compared to meals based on cereals or legumes. [14] Highly variable *in vitro* availability of iron, ranging from 6 to 44% in 13 crude leafy vegetables in India, was also reported [15], due to interactions between the components in the leaves studied. In an *in vivo* iron absorption study of a typical Burkinabe meal, iron absorption from maize paste eaten with a traditional leafy vegetable sauce with amaranth leaves was compared to maize paste plus iron-improved sauces containing extra leaves of amaranth or Jew's mallow (also called jute). [16] However in that study, despite higher iron content per serving in the iron-improved sauces, the increase in the proportion of leafy vegetables in the sauce compared to the traditional recipe did not provide additional bioavailable iron.

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Table 1: Composition of the sauces

Ingredients	Amaranth sauce	Jew's mallow sauce
	(g of ingredient per 500 g of sauce)	
Amaranth leaves	182.2	0
Jew's mallow leaves	0	175.3
Fresh tomato	98.6	98.6
Water	81.2	88.1
Fresh onion	54.8	54.8
Tomato puree	26.2	26.2
Groundnut paste	17.0	17.0
Peanut oil	16.3	16.3
Dried fish	14.1	14.1
Soumbala	4.6	4.6
Stock cube	3.2	3.2
Coarse salt	1.1	1.1
Solid potash	1.0	1.0

The high concentration of phenolic compound in the leafy vegetables was assumed to impair iron bioavailability in these dishes. Moreover, the ascorbic acid content - known to be an iron-absorption enhancer - was close to zero in the sauces, due to the long cooking required to soften the texture of the leaves. As long as most people in Burkina Faso and other sub-Saharan countries cannot afford to include animal source foods in their diet, the fortification of sauces with micronutrients could be a temporary solution. Ferrous sulfate and sodium iron EDTA are often used as fortificants. [17] Ferrous sulfate is the most frequently used and the cheapest, water-soluble iron fortificant. EDTA is known as a strong chelator of divalent cations and then protects iron against chelation by iron-absorption inhibitors. [18] Therefore, sodium iron EDTA is recommended in high-phytate foods, such as cereal based products because its absorption is greater than other iron fortificants. In food with low phytate content, its absorption is equivalent but it has other technical advantages. In this context, the aim of our study was to assess the bioaccessibility of iron and zinc in the same amaranth and Jew's mallow sauces as in the previous study[16], alone or mixed with a portion of maize paste, as usually consumed. The effect of fortification of these sauces with either iron sulfate or sodium iron EDTA on iron and zinc bioaccessibility was analyzed.

2 Materials and methods

Dish preparation

LV sauces and maize paste were prepared as previously described[19,20] with some modifications described below.

Sauce ingredients

Approximately 5 kg of leaves of Amaranth (*Amaranthus cruentus*) and Jew's mallow (*Corchorus olitorius*) were purchased from local gardeners in Ouagadougou (Burkina Faso). They were carefully washed three times with tap water and once with deionized water to remove any trace of soil or dust that could lead to iron contamination, before freezing. Leaves were kept frozen until analysis and preparation of the sauces. Stock cubes, potash, groundnut paste, dried fish, *soumbala* (fermented beans used as a relish) were purchased in a local market in Ouagadougou and kept frozen until needed. Tomato puree, fresh tomatoes and onion were purchased in a local market in Montpellier just before use. Ingredients from the same batch were used for each batch of sauce.

Sauce preparation

Sauces were prepared in the laboratory according to a standardized recipe to obtain sauces with 20% dry matter using the same proportion of ingredients except for the type of leaves (Table 1). The iron compounds used for the fortification of amaranth and Jew's mallow sauces were ferrous sulfate and sodium iron ethylenediaminetetraacetate (NaFeEDTA) kindly provided by DSM (Heerden, The Netherlands). The levels of fortification were chosen to target a total iron content of 8 mg (level L1) and 16 mg (level L2) per 100 g of sauce taking into account mean intakes of respectively 166 and 66 g of sauce per meal by women of childbearing age and young children in Burkina Faso[20] and a targeted contribution to iron recommended nutrient intake (RNI) of 50%. Corresponding quantities of iron compounds were carefully weighed, added to the diluted sauces contained in a large bottle, and homogenized on a shaking table for 1 h at 4 °C. Aliquots (50 mL) of fortified sauces were then frozen until use.

Preparation of « tô » cereal paste

A cereal-based thick paste whose local name is « tô » was prepared from decorticated maize flour with a final dry matter content of 22%-23% according to the traditional recipe from Burkina Faso but with quantities adapted for laboratory use. The phytate content of this flour was low (around 130 mg phytate/100g DM[16]).

Preparation of dishes of tô and sauces ("whole dishes")

Mixes of sauces plus tô, representing typical dishes eaten in sub-Saharan Africa and called "whole dish" in this paper, were prepared at a proportion of 2:1 on a fresh basis, as traditionally consumed and used for micronutrient bioaccessibility measurements. Mixes were homogenized with an Ika Ultra-Turrax T25 (Staufen, Germany) before use.

Analytical methods

DM contents were determined by oven drying at 105 °C to constant weight.

Iron and **zinc** were extracted with a closed-vessel microwave digestion system (ETHOS-1, Milestone, Italy) and analyzed using a Perkin-Elmer AAnalyst 800 Atomic Absorption Spectrometer (Waltham, USA). Standard reference materials BCR-679 White Cabbage and BCR-191 Brown Bread (IRMM- Institute for Reference Materials and Measurements, European Commission) were used as controls with iron SpectrAA measurements. The coefficients of variation obtained with these two reference materials were 5.74 and 5.62% respectively, with distances from the reference value of -1.24% in the case of white cabbage and -4.62% in the case of brown bread.

Iron and zinc bioaccessibility, also called availability[22] was determined using dialysability used to obtain information about digestion of the food and release of the minerals from the food matrix. Iron and zinc bioavailability depends on both food and host factors, and thus its assessment requires the use of *in vivo* methods on humans, to consider each step involved[21]: (i) digestion and release of nutrients from the foods, (ii) uptake followed by (iii) absorption into the enterocytes, (iv) retention and (v) transport to tissues or storage sites for use and/or storage. As preliminary screening tools, *in vitro* methods including solubility, dialysability and simulated gastrointestinal digestion or Caco-2 cells have been

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Table 2: Bioaccessibility of iron and zinc in amaranth and Jew's mallow sauces or in the whole dishes comprising sauce plus tô

Sauces	Total mineral (mg/100g)	Dialysable fraction (%)	Soluble fraction (%)	Insoluble fraction (%)	Dialysable fraction (mg/100g)
Fe					
Amaranth	3.08 ± 0.21	8.1 ± 1.4 ^{b}	13.3 ± 1.1 ^{b}	78.7 ± 1.0 ^{a}	0.25 ± 0.05 ^{b}
Jew's mallow	3.06 ± 0.24	0.9 ± 0.7 ^{a}	3.1 ± 0.3 ^{a}	96.0 ± 1.0 ^{b}	0.03 ± 0.02 ^{a}
Amaranth + tô	1.07 ± 0.09	6.8 ± 1.7 ^{b}	14.7 ± 1.0 ^{b}	78.6 ± 2.7 ^{a}	0.07 ± 0.02 ^{a}
Jew's mallow + tô	1.12 ± 0.00	2.1 ± 0.3 ^{a}	3.4 ± 0.6 ^{a}	94.5 ± 0.3 ^{b}	0.02 ± 0.00 ^{a}
Zn					
Amaranth	0.60 ± 0.00	58.8 ± 11. 3 ^{b}	0.0 ± 0.0 ^{a}	41.2 ± 3.7 ^{a}	0.38 ± 0.07 ^{c}
Jew's mallow	0.51 ± 0.09	32.0 ± 6.9 ^{a}	1.3 ± 1.6 ^{a,b}	64.9 ± 4.9 ^{b}	0.17 ± 0.05 ^{b}
Amaranth + tô	0.11 ± 0.01	46.5± 2.8 ^{a,b}	4.6 ± 4.2 ^{b}	43.2 ± 8.0 ^{a}	0.05 ± 0.04 ^{a}
Jew's mallow + tô	0.10 ± 0.00	46.3 ± 9.0 ^{a}	0.0 ± 0.0 ^{a}	58.5 ± 11.6 ^{a,b}	0.05 ± 0.01 ^{a}

Results are means of 3 measurements ± standard deviation; values in one column for the same mineral with different superscript letters are significantly different ($p < 0.05$).

developed to compare different foods. [22] These methods are simpler, less expensive and give models of iron and zinc absorption that can be used to develop hypothesis. We used an appropriate *in vitro* procedure[23] with modifications and including first the determination of the titratable acidity of samples[24] and a simulated digestion. Micronutrient bioaccessibility was measured at least in triplicate on samples of unfortified sauces, fortified sauces, and whole dishes prepared "as eaten" i.e. freshly cooked in the manner they are normally consumed[22]. Samples were homogenized with ultrapure water to obtain a DM content of 9-10% in sealed glass flasks and brought to 37 °C in a water bath. Alpha-amylase (20 µL) from *Bacillus licheniformis* (Sigma A-3403-1MU) was added to the samples, which were then incubated at 37 °C for 5 min. The pH was adjusted to 2.0 with 1 M HCl; 1 mL of pepsin solution was then added (Sigma, P-7000, 14900 u/mL in 0.1 M HCl) and the samples were incubated horizontally for 1 h at 37 °C in a shaking water bath. Aliquots (40 g) of each pepsin-digested sample were then transferred in separate large tubes for the intestinal digestion. To gradually increase the pH to mimic intestinal digestion, a dialysis bag (Spectra/por I dialysis tubing, MCO 12-14kDa) containing 20 mL of the PIPES (piperazine-N,N'-bis-[2- ethanesulfonic acid] sodium salt) buffer (Sigma, P-3768) of the previously determined molarity from titratable acidity results, was introduced into each large tube and incubated at 37 °C for 30 min to reach pH 6.7. Five mL of enzyme solution, containing pancreatin (Sigma, P1750, 1.85 mg/mL) and bile extract solution (Sigma, B8631, 11 mg/mL in 0.1M $NaHCO_3$), were then added, and the samples were incubated horizontally for 2 h at 37 °C in a shaking water bath. During incubation, dialyzable iron was diffused into the dialysis bag so that the same concentration was reached on both sides of the membrane. The dialysis bags were then removed and washed with pure water. Their contents (the dialysates) were weighed. The digestion mixtures remaining in the tubes were centrifuged at 10 000 g for 15 min at 4 °C to separate the insoluble and soluble iron fractions, respectively, in the pellet and supernatant. Thus, the sum of dialyzed, soluble non-dialyzed and insoluble fractions should be equal to the total amount of mineral in the sample before digestion.

The sum of dialysable, soluble non-dialysable (SND) and insoluble fractions should thus be equal to the total amount of iron in the sample before digestion. Each fraction was calculated on the basis of the total iron or zinc recovered at the end of digestion:

$$\text{Dialysable Fe or Zn \%} = C_D (W_D + W_S)/ (C_D W_D + C_S W_S + C_I W_I) \times 100 \quad (1)$$

$$\text{SND Fe or Zn \%} = W_S (C_S - C_D)/ (C_D W_D + C_S W_S + C_I W_I) \times 100 \quad (2)$$

$$\text{Insoluble Fe or Zn \%} = W_I (C_I)/ (C_D W_D + C_S W_S + C_I W_I) \times 100 \quad (3)$$

where C_D, C_S and C_I are iron concentrations (µg/100 g) and W_D, W_S and W_I are the weights (g) of the dialysate, supernatant, and pellet, respectively. The iron contents of each fraction were analyzed as described above. Results are expressed as a percentage (fractional iron) of the total iron in the food.

Statistical analysis

Analyses were performed in triplicate. Values were averaged. Data were subjected to analysis of variance (ANOVA) and Fischer's least significant difference tests were used to compare means at the 5% significance level, using the software Statgraphics Plus version 5.1.

3 Results

Iron and zinc bioaccessibility in the sauces and in the whole dishes

Total iron contents in amaranth and Jew's mallow sauces were similar and 5 to 6 times higher than total zinc contents in both sauces, which were very low (Table 2). In the whole dishes, the addition of tô resulted in a decrease in iron and zinc contents. This was due to a "dilution" effect of the already low iron and zinc contents in maize tô (0.15 and 0.10 mg/ 100 g respectively).

In the amaranth sauce, dialysable iron content was 0.25 mg/100 g, which represented 8.1% of total iron. In the whole dish prepared with this sauce, the behavior of iron was not significantly modified: the percentage of dialysable iron was slightly but not significantly

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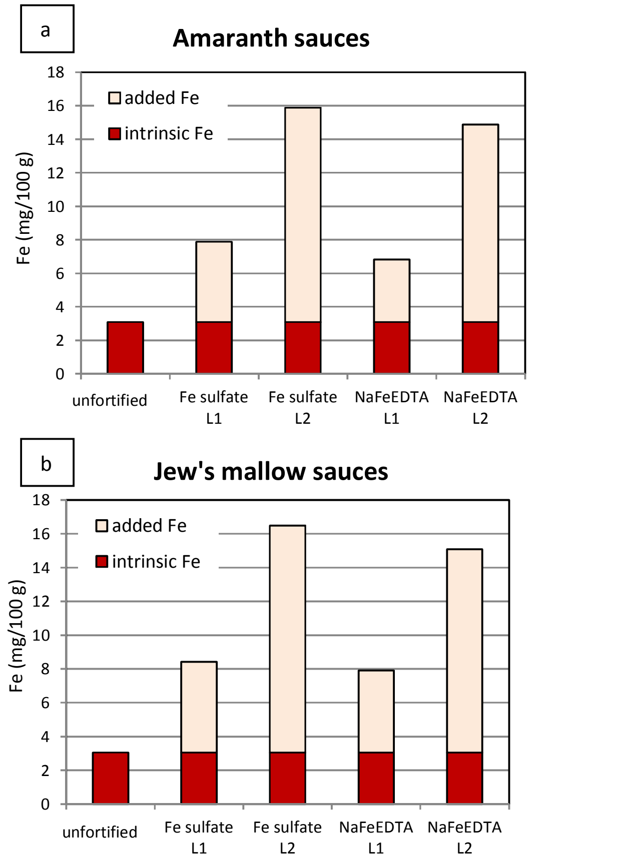
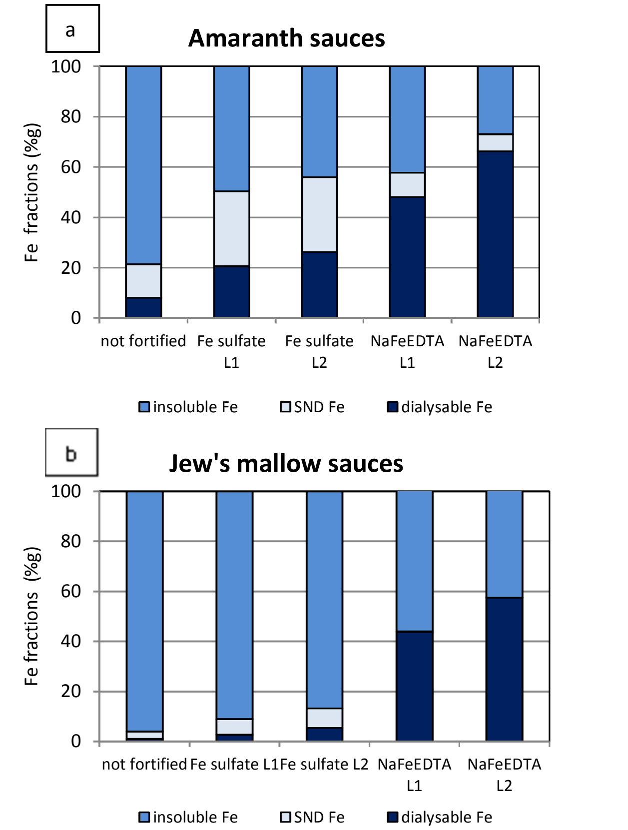


Figures 1a and 1b: Distribution of native and added iron in amaranth and Jew's mallow sauces fortified (or not) with NaFeEDTA or iron sulfate. L1 and L2 are two levels of fortification, L1 = 8 mg of iron/100 g of sauce; L2 = 16 mg of iron/100 g of sauce.



Figures 2a and 2b: Distribution of the dialysable, soluble or insoluble iron of amaranth and Jew's mallow sauces fortified (or not) with NaFeEDTA or iron sulfate after *in vitro* digestion. L1 and L2 are two levels of fortification: L1 = 8 mg of iron/100g of sauce; L2 = 16 mg of iron/100g of sauce. SND Fe = Soluble Non Dialysable iron.

lower than in the sauce, giving a dialysable iron content about 3.4 times lower. The percentages of soluble and insoluble iron were also similar, as if the difference between the amaranth sauce and the whole dish was only due to the dilution effect of adding tô. In the Jew's mallow sauce, the dialysable iron content was 0.03 mg/100 g of sauce, i.e. much lower of that of the amaranth sauce, even though total iron content was similar. Indeed, the dialysability of iron was 10 times lower than in amaranth sauce. The soluble non dialysable iron fraction was also much lower, and the insoluble iron fraction was significantly higher in the Jew's mallow sauce. When mixed with tô in a whole dish, the percentage of dialysable iron was slightly but not significantly higher than in the Jew's mallow sauce alone. Their dialysable iron contents were similar, as if the higher dialysability compensated for the "dilution" effect of tô. Soluble and insoluble iron percentages also resembled those in the amaranth sauce and whole dish. It thus appears that maize tô hardly influences the amount of dialysable iron supplied by the Jew's mallow sauce.

Despite similar total zinc contents, the percentage and concentration of **dialysable zinc** were almost twice higher in the amaranth sauce than in the Jew's mallow sauce. Almost two-thirds of the total zinc in the amaranth sauce was dialysable, i.e., much higher than iron. In the whole dishes with amaranth or Jew's mallow sauces, the distribution in the different fractions was much the same, i.e. about half of total zinc was dialysable, and the remaining zinc was insoluble. The percentage of soluble zinc in the sauces was negligible and in the whole dishes, was less than 5%. Dialysable zinc content was 3.6 to 7 times lower in the whole dishes due to a simple dilution effect of mixing the sauce with the tô.

Iron and zinc bioaccessibility in fortified sauces

Fortification of amaranth or Jew's mallow sauces with NaFeEDTA or Fe sulfate led to ***total iron*** contents that are consistent with targeted values (Figs. 1a and 1b): in both sauces, the total iron contents obtained with both fortificants did not differ significantly, whatever the fortification level. Added iron, i.e. iron supplied through fortification, represented respectively 60% and 80% of total iron in the sauces with L1 and L2 fortification levels. As NaFeEDTA or Fe sulfate did not contain zinc, the total zinc content of the fortified sauces remained unchanged after fortification.

The ***percentage of dialysable iron*** in the fortified amaranth sauces was significantly higher than that in the unfortified sauce (Figs. 2a and 2b): at levels L1 and L2, it was respectively 6 and 8 times higher with NaFeEDTA, and 2.5 and 3.2 higher with iron sulfate. The marked effect of NaFeEDTA, and to a lesser extent that of iron sulfate, on iron dialysability was also measured in fortified Jew's mallow sauces: at both levels L1 and L2, the percentage of dialysable iron was respectively 48 and 64 times higher with

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Table 3: Bioaccessibility of *iron* in fortified amaranth and Jew's mallow sauces

	Sauces	Total Fe (mg/100g)	Dialysable Fe (mg/100g)	Soluble Fe (mg/100g)	Insoluble Fe (mg/100g)
Amaranth	**Unfortified**	3.08 ± 0.21	0.25 ± 0.05 ^{a}	0.41 ± 0.01 ^{a}	2.42 ± 0.19 ^{a}
	Fe sulfate L1	7.87 ± 0.28	1.62 ± 0.14 ^{b}	2.34 ± 0.13 ^{d}	3.91 ± 0.21 ^{b}
	Fe sulfate L2	15.88 ± 1.19	4.17 ± 0.29 ^{c}	4.72 ± 0.19 ^{e}	6.97 ± 0.75 ^{c}
	NaFeEDTA L1	6.82 ± 1.49	3.29 ± 0.83 ^{c}	0.66 ± 0.14 ^{b}	2.87 ± 0.58 ^{a}
	NaFeEDTA L2	14.87 ± 1.77	9.87 ± 1.50 ^{d}	1.02 ± 0.11 ^{c}	3.98 ± 0.16 ^{b}
Jew's mallow	**Unfortified**	3.06 ± 0.24	0.03 ± 0.02 ^{a}	0.09 ± 0.01 ^{b}	2.93 ± 0.24 ^{a}
	Fe sulfate L1	8.42 ± 0.50	0.22 ± 0.01 ^{a,b}	0.52 ± 0.01 ^{c}	7.68 ± 0.51 ^{d}
	Fe sulfate L2	16.48 ± 0.14	0.09 ± 0.01 ^{b}	1.28 ± 0.04 ^{d}	14.31 ± 0.21 ^{e}
	NaFeEDTA L1	7.91 ± 1.44	3.46 ± 0.66 ^{c}	0.02 ± 0.04 ^{a}	4.55 ± 0.70 ^{b}
	NaFeEDTA L2	15.07 ± 0.92	8.67 ± 0.70 ^{d}	0.00 ± 0.00 ^{a}	6.57 ± 0.28 ^{c}

Results are means of 3 measurements ± standard deviation; values in one column for the same sauce with different superscript letters are significantly different ($p < 0.05$). L1 and L2 are two levels of fortification, L1 = 8 mg of iron/100 g of sauce; L2 = 16 mg of iron/100 g of sauce

NaFeEDTA, and 3 and 6 times higher with iron sulfate. Compared to their respective unfortified sauces, the percentages of ***soluble iron*** were significantly lower in amaranth and Jew's mallow sauces fortified with NaFeEDTA and higher in the sauces fortified with iron sulfate. Concomitantly, all fortified sauces had percentages of ***insoluble iron*** below that of unfortified ones.

Fortification of sauces had also a huge effect on ***dialysable iron content*** that differed greatly depending on the nature of the fortificant (Table 3). In amaranth sauce fortified with Fe sulfate at fortification levels L1 and L2, dialysable iron content was respectively 7 and 17 times higher than in the unfortified sauce.

This increase was greater than would be expected due to the total increase in iron caused by the increase in iron dialysability. When the fortificant was NaFeEDTA, this increase was even higher, reaching 13 and 40 times that in unfortified amaranth sauce. In the Jew's mallow sauce, the increases were respectively 3 and 8 at levels L1 and L2 in the sauces fortified with Fe sulfate and 128 to 321 in those fortified with NaFeEDTA. In Jew's mallow sauce fortified with Fe sulfate, the increase in dialysable iron was not as great as in amaranth sauce, again underlining the marked effect of the Jew's mallow leaf matrix. However, when NaFeEDTA was used as fortificant, the dialysable iron content increased dramatically and proportionally to the level of fortification. In both amaranth and Jew's mallow sauces, whatever the level of fortification, the soluble non-dialysable iron content was less in the sauces fortified with NaFeEDTA than in the sauces fortified with Fe sulfate, in contrast to dialysable iron content. Similar results were obtained for insoluble iron content. The increase in insoluble iron content was generally much lower than that of dialysable iron in the sauces, showing that iron fortification mainly acts by increasing the concentration of dialysable iron, and consequently by increasing the bioaccessibility of this micronutrient in the sauces, showing that NaFeEDTA is much more effective than iron sulfate for this purpose.

Total zinc contents were similar in all sauces, fortified or unfortified (Table 4), due to the absence of zinc in the fortificants used. Percentages of dialysable zinc depended on both the leaf matrix and the type of fortificant. When the amaranth sauces were fortified with iron sulfate, zinc dialysability decreased significantly. Surprisingly, in Jew's mallow sauces, zinc dialysability was significantly higher in sauces fortified with NaFeEDTA than in sauces fortified with Fe sulfate or not fortified. As a result, dialysable zinc contents were slightly higher in the Jew's mallow sauces fortified with NaFeEDTA whatever the level of fortification. In both leafy vegetable sauces fortified with NaFeEDTA, the percentage of soluble zinc increased, whereas it was close to zero or zero in the sauces fortified with iron sulfate or not fortified. The percentages of insoluble zinc decreased proportionally.

4 Discussion

In our study on sauces or on whole dishes of sauce and tô, we showed that the difference in iron bioaccessibility between the amaranth sauce and the corresponding whole dish was probably due to the dilution caused by adding tô. We also showed that iron was much more dialysable and soluble in amaranth sauce than in Jew's mallow sauce whereas the percentage and concentration of insoluble iron were significantly higher in the Jew's mallow sauce. That is why the dilution effect due to the addition of tô led to a decrease in iron bioaccessibility in the whole dish made from tô + amaranth sauce compared to the sauce alone, while it increased in the case of Jew's mallow. This difference between the two leaf species may be related to their biochemical composition, particularly of their **fibres**. It is known that Jew's mallow leaves are rich in water soluble mucilage that give rise to viscous hydrocolloid solutions. [25] Indeed, the Jew's mallow sauce is characterized by its slimy texture, which can be explained by this high water soluble mucilage content. Mucilage is a non-starch polysaccharide that contains uronic acids, mainly glucuronic and galacturonic acid. [25] As pectin also contains uronic acids that have mineral-binding properties due to their carboxyl groups[26], we hypothesize that the mucilage in Jew's mallow sauces binds divalent cations such as iron or zinc, which may explain their lower mineral bioaccessibility compared to amaranth sauce. As the pKa of glucuronic acid ranged between 2 and 4, the carboxyl groups may be deprotonated as the pH increases during digestion and then may bind part of the iron, as shown for pectin[26], a polysaccharide rich in polygalacturonic acid. However, such a difference between amaranth and Jew's mallow sauce was not found in an in vivo absorption study[16] in which both sauces exhibited the same absorption rate of 4.9%. As reviewed recently[27], the negative effects of fibers on mineral bioavailability observed *in vitro* are not always confirmed *in vivo*. **Other chelating factors** may also have an effect on the bioaccessibility of iron and zinc in the amaranth and Jew's mallow sauces. In the previously cited study[16], the authors explained that the low iron absorption level obtained for the improved sauces containing extra leaves could be due to their higher content in phenolic compounds, known to inhibit iron absorption, and that may have played the role of a

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Table 4: Bioaccessibility of *zinc* in fortified amaranth and Jew's mallow sauces

	Sauces	Total Zn (mg/100g)	Dialysable Zn (%)	Soluble Zn (%)	Insoluble Zn (%)	Dialysable Zn (mg/100g)
Amaranth	**Unfortified**	0.60 ± 0.00	62.8 ± 12. 1 ^{b}	0.0 ± 0.0 ^{a}	44.0 ± 3.9 ^{c}	0.38 ± 0.07 ^{b}
	Fe sulfate L1	0.53 ± 0.06	55.0 ± 8.0 ^{a}	1.2 ± 1.1 ^{a}	45.5 ± 3.1 ^{c}	0.29 ± 0.03 ^{a}
	Fe sulfate L2	0.63 ± 0.03	53.6 ± 2.5 ^{a}	0.8 ± 1.3 ^{a}	46.9 ± 0.4 ^{c}	0.33 ± 0.02 ^{a,b}
	NaFeEDTA L1	0.48 ± 0.03	78.6 ± 1.9 ^{b}	12.3 ± 1.0 ^{b}	9.1 ± 3.9 ^{b}	0.38 ± 0.03 ^{b}
	NaFeEDTA L2	0.46 ± 0.09	81.0 ± 3.4 ^{b}	15.3 ± 1.0 ^{b}	3.6 ± 3.4 ^{a}	0.38 ± 0.02 ^{b}
Jew's mallow	**Unfortified**	0.51 ± 0.09	34.2 ± 7.4 ^{a}	1.3 ± 1.7 ^{a}	64.9 ± 5.2 ^{b}	0.17 ± 0.05 ^{a}
	Fe sulfate L1	0.50 ± 0.03	38.4 ± 3.4 ^{a}	1.6 ± 1.7 ^{a}	61.1 ± 2.6 ^{b}	0.19 ± 0.03 ^{a}
	Fe sulfate L2	0.50 ± 0.01	37.8 ± 1.9 ^{a}	0.1 ± 0.2 ^{a}	62.8 ± 0.8 ^{b}	0.19 ± 0.02 ^{a}
	NaFeEDTA L1	0.40 ± 0.11	70.9 ± 6. 4 ^{b}	18.6 ± 3.8 ^{b}	10.5 ± 2.6 ^{a}	0.28 ± 0.06 ^{b}
	NaFeEDTA L2	0.43 ± 0.08	73.4 ± 3.9 ^{b}	13.8 ± 4.4 ^{b}	12.7 ± 3.1 ^{a}	0.31 ± 0.06 ^{b}

Results are means of 3 measurements ± standard deviation; values in one column for the same sauce with different superscript letters are significantly different ($p < 0.05$). L1 and L2 are two levels of fortification, L1 = 8 mg of iron/100 g of sauce; L2 = 16 mg of iron/100 g of sauce

limiting factor. But additional information on polyphenol types of the two leaf species would be necessary to confirm this hypothesis. In the same study, the phytate contents of the whole dishes were analogous and low. It can thus be assumed that phytates are not responsible for the differences in the available iron fraction between the two species of leafy vegetable. In the present study, the zinc content of both types of leaf and their corresponding sauce were low, which was not surprising as leafy vegetables are known to be a poor source of zinc. [28] Dialysable zinc contents were also lower in the whole dishes than in the corresponding sauces, whatever the leaf. The initial zinc content in the sauces was low, and then, the dialysable zinc content in the whole dishes made of sauce plus tô was also quite low. Concerning enhancers of iron or zinc bioaccessibility, the effect would be no different as a function of the leaf species because the sauces have the same ingredients (for example the same animal protein content) and even if ascorbic acid content is relatively high in the leaf, the long cooking would probably have entirely destroyed it.

Whatever the leaf species used to prepare the sauces, iron dialysability was improved by fortification but at varying intensity depending on the nature of the **fortificant**. Iron fortification mainly acts by increasing dialysable iron, NaFeEDTA being more effective than iron sulfate for this purpose. This result is consistent with results of absorption studies in Central America[29], who showed that iron was better absorbed from NaFeEDTA than from iron sulfate when these fortificants were given in an aqueous solution or with a standard meal including cereal, beans, etc. It can be hypothesized that when iron sulfate is used, the whole pool of iron, either native or added is strongly affected by the chelating factors present in the food matrix. Conversely, when NaFeEDTA is used, it is as if EDTA exerts a protective effect against the chelation of native or added iron by compounds of the food matrix. This result is in good agreement with results obtained by other authors[30], who showed that iron absorption from the Fe(III)-EDTA complex is only slightly or not at all affected by the presence of vegetable foods.

Zinc bioaccessibility was also improved by iron fortification whit NaFeEDTA and particularly in Jew's mallow sauces. Interactions between zinc and NaFeEDTA are stronger than between zinc and the food matrix, as shown by the increase in dialysable and soluble zinc fractions. Others authors showed that -depending on its concentration- NaFeEDTA could improve the absorption of non-heme iron[29]. In a bioavailability study on rats including a high phytic acid diet[31], it was shown that Zn supplied as an EDTA disodium salt has a higher bioavailability than other commonly used Zn salts. In an *in vivo* study, it was also shown that NaFeEDTA added to diets with low mineral bioavailability could increase zinc absorption. [32] EDTA was also shown to increase zinc absorption in humans from zinc sulfate fortified maize and sorghum. [33] Like iron, EDTA could have a positive effect on zinc absorption by protecting it against the formation of complexes with some inhibitory factors like phenolic compounds. NaFeEDTA is also known to exchange completely with intrinsic iron in the lumen of the gut. [30]

Conclusions

This study shows that the bioaccessibility of iron and zinc is regulated by interactions between the food matrix, either leafy vegetables or the maize-based component and the mineral, and between the mineral and the fortificant. Due to their varying effects on iron bioaccessibility that we refer to as the "matrix effect", a more detailed analysis of the biochemical composition of the different leaf types should now be performed to explain the interactions between the matrices and iron in leafy vegetables. It would also be interesting to further investigate the protective mechanisms of EDTA on iron and zinc absorption in these complex slimy matrices, rich in mucilaginous polysaccharides. Our results also show that iron fortification would be an efficient way to greatly increase the available iron content of leafy vegetable sauces and that for this purpose the use of NaFeEDTA should be preferred. But whatever the fortificant, sensory analysis should also be performed to assess the acceptability of the fortified sauces by the consumers, due to potential changes in color or taste.

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Acknowledgements

Supported by the INSTAPA project that received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration.

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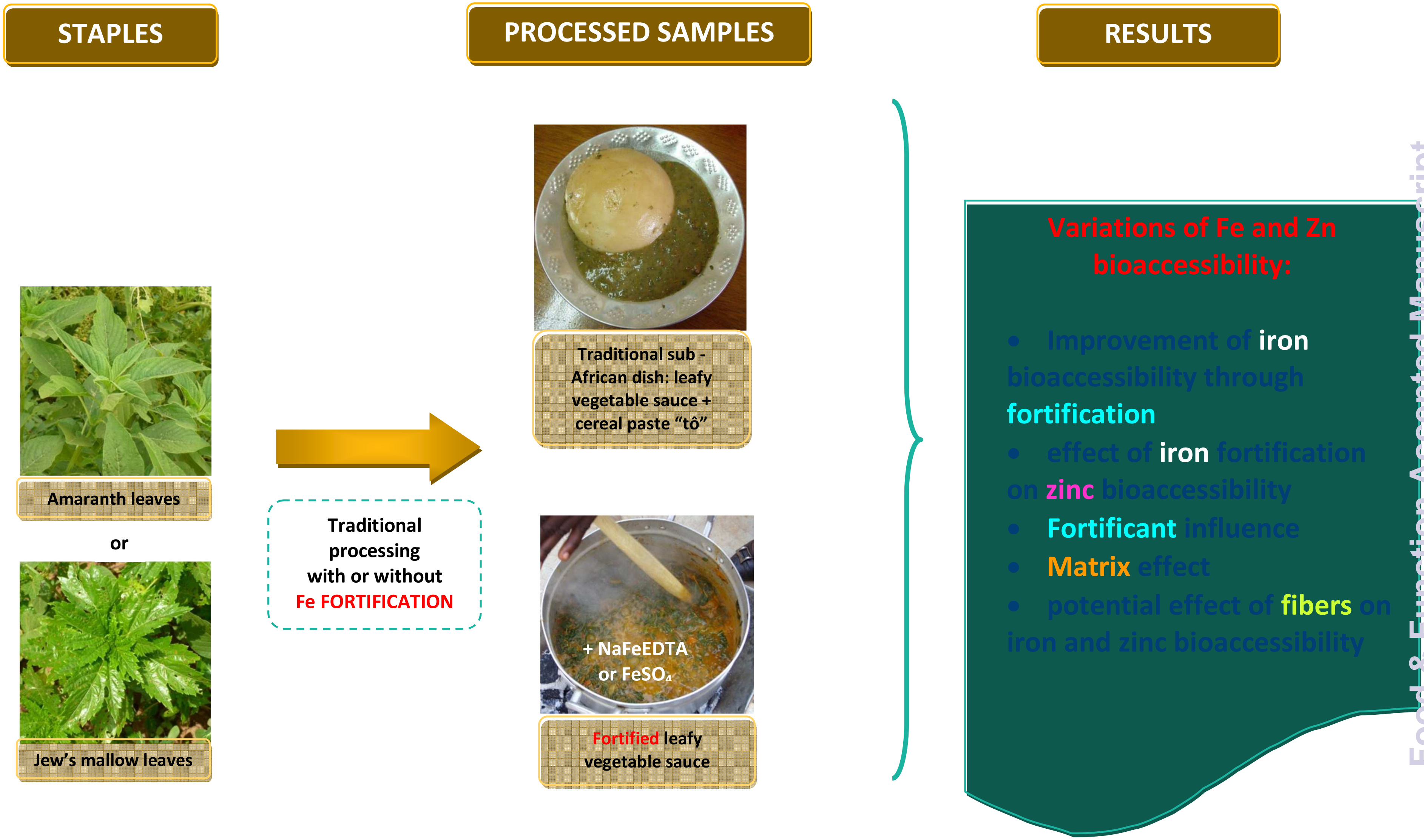

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