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ScienceDirect
Food Chemistry 101 (2007) 419–427
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

Oxidative activity and dialyzability of some iron compounds under conditions of a simulated gastrointestinal digestion in the presence of phytate

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Received 7 June 2005; received in revised form 20 October 2005; accepted 29 December 2005

Abstract

The oxidative activity of some iron compounds was compared under conditions of gastrointestinal digestion and correlated with iron solubility and dialyzability. Ferric chloride, NaFeEDTA, ferrous bis-glycinate, ferrous gluconate, or ferrous lactate, were mixed with water or a phytate solution or a phytate solution treated with phytase and digested in vitro. The dialyzable and the non-dialyzable fractions of the digests were analyzed for oxidative activity on linoleic acid liposomes and iron solubility and dialyzability. In all dialyzable and non-dialyzable fractions, oxidative activity was variable in water ($P < 0.05$), lower and similar for all iron compounds in phytate ($P < 0.05$), higher and variable in phytate solutions treated with phytase ($P < 0.05$). In most digests, ferric chloride was least active, followed by ferrous lactate, NaFeEDTA and ferrous bis-glycinate. There was no correlation between oxidative activity and iron dialyzability or solubility. These results point to variable oxidative properties that iron compounds may potentially exhibit in the lumen.

Keywords: Iron dialyzability; Fortification; Oxidative activity; Phytate; Phytase

1. Introduction

Primary prevention of iron deficiency may be achieved through an iron adequate diet (Anonymous, 1999; Centers for Disease Control, 1998; Lynch and Stoltzfus, 2003; Ziegler and Fomon, 1996). One approach to increase dietary iron intake is iron fortification of selected foods (Hurrell, 2002). Various iron compounds, including ferrous sulfate, NaFeEDTA, ferrous lactate, iron pyrophosphate and ferrous bis-glycinate, have been developed to meet nutritional, technological and organoleptic needs. These iron compounds exhibit variable bioavailability. The search of compounds that provide highly bioavailable iron and do not affect the organoleptic properties of the fortified food remains a subject of continuous study. In addition to this research challenge, recent reports associate dietary iron with potential increase of lipid peroxidation in the gastrointestinal environment (Babbs, 1990; Lund, Fairweather-Tait, Wharf, & Johnson, 2001; Lund, Wharf, Fairweather-Tait, & Johnson, 1999). Presumably, iron that is not absorbed remains in the intestinal lumen where it may catalyse oxidation processes, implicated in diseases of the gastrointestinal tract (Siegers, Bumann, Baretton, & Younes, 1988). Evidence in support or against this hypothesis is not yet concrete. The oxidative activity of different iron compounds including those used in iron fortification has not been evaluated. Lund et al. (2001) found in rats that chronic exposure to high levels of ferrous sulfate increased lipid peroxidation in the mucosa of the large intestine and that phytate exhibited an antioxidant effect in the lumen. Phytate, however, inhibits iron absorption; hydrolysis of phytate by phytase improves iron absorption

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doi:10.1016/j.foodchem.2005.12.037

(Davidsson, 2003; Hurrell, Reddy, Juillerat, & Cook, 2003) but may decrease the antioxidant effect of phytate (Porres et al., 1999). It follows that the oxidative activity of iron compounds may depend on a variety of factors, such as the presence of iron chelators that derive from foods.

The objective of this study was (a) to test in vitro the hypothesis that different iron compounds exert variable oxidative activity under gastrointestinal conditions and that phytate or phytate treated with phytase may modify the oxidative activity of the iron compounds and (b) to correlate the oxidative activity of the iron compounds with their solubility and dialyzability under conditions of in vitro digestion. Mixtures of selected iron compounds (NaFeEDTA, ferrous bis-glycinate, ferrous gluconate, and ferrous lactate) and water or a phytate solution or a phytate solution treated with phytase were subjected to an in vitro digestion procedure. The oxidative activity of the digests was evaluated on linoleic acid liposomes by the TBARS method. The solubility and dialyzability of iron was determined and correlated with oxidative activity measured in the in vitro digests.

2. Materials and methods

2.1. Materials

The iron compounds tested in this study were NaFeEDTA (Ferrazone®, 13.2% iron according to the manufacturer), ferrous bis-glycinate (Ferrochel®, 20.1% iron according to the manufacturer), ferrous gluconate (Ferrous Gluconate®, 12.5% iron according to the manufacturer), ferrous lactate (Ferrous L-Lactate®, 24% iron according to the manufacturer). All iron compounds was a generous donation from the Research and Development Department of Delta Dairy SA, Athens, Greece. NaFeEDTA was kindly donated from Akzo Nobel, Herkenbosch, The Netherlands.

The materials used in the in vitro digestion experiment were the following. Pepsin was a porcine pepsin preparation, suspended in 0.1 M HCl at 4 g/100 mL in 0.1 M HCl. Pancreatin/bile mixture was a porcine pancreatin (0.2 g) and a bile extract (1.2 g) suspended in 100 mL of 0.1 M $NaHCO_3$. PIPES buffer, 0.15 M PIPES (piperazine-*N,N′*-bis[2-ethane-sulfonic acid] disodium salt), was adjusted to pH 6.3 using concentrated HCl. HEPES buffer, 0.3 M HEPES (*N*-2-hydroxyethyl-piperazine-*N′*-2-ethane-sulfonic acid sodium salt) pH 9.5, was used without pH adjustment. Protein precipitant solution (reducing) was 100 g trichloroacetic acid, 50 g hydroxylamine monohydrochloride and 100 mL concentrated HCl per 1 L of water. Protein precipitant solution (non-reducing) was prepared as the reducing solution except that the hydroxylamine monohydrochloride was not added. Ferrozine chromogen solution (5 mg/mL) was of ferrozine (3-(2-pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4 triazine, disodium salt). Spectrapore® I dialysis tubing with a molecular weight cut-off of 6000–8000 (Spectrum Laboratories, Rancho Dominguez, CA, USA) was cut into 25 cm lengths and soaked in water for at least 1 h prior to use and stored in 0.15 M PIPES buffer until use. An iron atomic absorption standard solution (1000 ppm) was used for the generation of a standard curve for the spectrophotometric determination of iron.

All chemicals and enzymes were purchased from Sigma-Aldrich. Double distilled, deionised water was used throughout the experiment. All glassware was washed, soaked overnight in 1 N HCl and rinsed with distilled deionised water.

2.2. Sample preparation

Sodium phytate 0.002 g/mL in glycine–HCl 0.1 M pH 2.5 was incubated with 1.25 mg/mL or 0.625 mg/mL phytase preparation (crude, from *Aspergilus ficuum*, 3.5 U/mg) at 37 °C for 30 min. Sodium phytate (0.002 g/mL) was incubated together with the samples treated with phytase. At the end of the incubation period, the hydrolysis of phytate was determined as in Shimizu (1992). A 20 mL aliquot of the incubated phytate solution or of the phytate solution treated with phytase was mixed with iron to a final concentration of 0.2 mM. Samples with iron but no phytate and controls with no iron were also prepared. All samples and controls were digested in vitro as described below.

2.3. Experimental protocols

2.3.1. In vitro digestion

The digestion process used was that described by Kapsokefalou and Miller (1991). This in vitro model simulates the gastrointestinal digestion by subjecting samples to incubation for 4.5 h at 37 °C, at different pHs, in the presence of peptic enzymes and by fractionating digests through the aid of a dialysis membrane. Briefly, samples of 20 mL at pH 2.5, were transferred in 120 mL screw-cap vials and placed in a shaking water bath maintained at 37 °C. The samples were incubated for 2 h in the presence of 1 mL pepsin suspension added to each sample. At the end of this incubation, the pH of the samples was gradually adjusted from 2.8 to 6 with the aid of a dialysis sac, filled with 20 mL of PIPES buffer, pH 6.3. The dialysis sac was immersed into the incubating samples. After 30 min, 5 mL of a pancreatin–bile salt mixture was added to the samples and the incubation continued for another 2 h. At the end of this incubation period, the dialysis sac was removed. The dialyzate, consisting of soluble compounds of low molecular weight, and the retentate, consisting of soluble and insoluble compounds of high molecular weight were collected. The pHs of the retentate and dialyzate were recorded and the contents of the dialysis bag were weighed. Dialyzates and retentates were centrifuged for 20 min at 10,000*g* and the supernatants were transferred for measurements of the iron concentration or for storage at −20 °C for measurement of linoleic acid peroxidation.

2.4. Linoleic acid peroxidation

Lipid peroxidation was assessed according to literature procedures (Matsingou, Petrakis, Kapsokefalou, & Salifoglou, 2003; Yamamoto, Niki, Kamyia, & Shimasaki, 1984): Linoleic acid was transferred under nitrogen into 0.30 M NaCl pH 7.4, to a final concentration of 5 mg/mL. The mixture was sonicated for 10 min under a nitrogen-saturated atmosphere, yielding a milky solution. A 0.5 mL quantity of the thawed retentates or dialyzates was placed in screw-capped tubes, followed by addition of 0.5 mL of the linoleic acid suspension. Blanks were prepared by replacing linoleic acid with 0.5 mL of 0.3 M NaCl pH 7.4. Controls were prepared by replacing the digests with water. All of the samples, blanks and controls were vortexed and incubated in a water bath at 37 °C for 2 h. At the end of the incubation period, assay samples were prepared by mixing solutions of 0.2 mL of each incubated mixture, 1.5 mL 20% acetic acid pH 3.5, 1.5 mL 0.8% thiobarbituric acid (TBA) and 0.8 mL water. Subsequently, all samples, blanks, and controls were vortexed and heated at 90 °C for 15 min. The pink chromogen, thus obtained, was extracted with 5 mL of 1-butanol and 1 mL water. The samples, blanks, and controls were subsequently centrifuged for 10 min at 2500*g*, and the absorbance of the organic layer was taken at 532 nm. Malondialdehyde (MDA) equivalents were calculated using linear regression analysis of a standard curve based on 1,1,3,3-tetraethoxypropane.

2.5. Iron analysis

Ferrous and total (ferrous and ferric) iron concentrations in the supernatants of the centrifuged dialyzates and retentates were measured using a modification of the ferrozine method proposed by Reddy, Chidambaram, Fonseca, and Bates (1986). Briefly, for total iron determination reducing protein precipitant solution (0.5 mL) was added to 1 mL aliquot of each supernatant of centrifuged dialyzate or retentate. For ferrous iron determination, non-reducing protein precipitant solution (0.5 mL) was added to 1 mL aliquot of each supernatant of centrifuged dialyzate and retentate. The samples were held overnight at room temperature. Subsequently, they were centrifuged for 10 min at 5000*g*. Aliquots of the supernatants (0.5 mL in duplicate) were transferred to separate tubes. Ferrozine solution (0.25 mL) and HEPES buffer (1.0 mL) were added to each tube. The absorbance of the samples was measured at 562 nm immediately after chromogen addition for the ferrous iron determination or 1 h after addition for the total iron determination. Sample iron concentrations were calculated from absorbance readings using a regression equation derived from data generated from an iron atomic absorption standard of 1000 ppm in the presence of the (reducing) protein precipitant solution.

Dialyzable ferrous iron, dialyzable total iron, soluble (dialyzable plus non-dialyzable) ferrous iron, and soluble total iron were expressed as percentages of the calculated total amount of iron in the treatment at the beginning of the digestion (Kapsokefalou, Alexandropoulou, Komaitis, & Politis, 2005).

2.6. Statistical analysis

Each individual sample was run in duplicate. Each experiment was repeated three times. Differences among samples containing selected iron compounds were tested with LSD test when ANOVA was significant. Means were concluded to be significantly different at 95% confidence interval, after testing for normality (Zar, 1999). Analysis of data was carried out with the program Statistica, version 5.1 (StatSoft, OK, USA).

3. Results

The oxidative potential of selected iron compounds subjected to an in vitro procedure that simulates the gastrointestinal processes was evaluated on linoleic acid liposomes (Fig. 1). Significant differences were observed between the selected iron compounds in the dialyzates and the retentates of the in vitro digests of water ($P < 0.05$). In the dialyzates and the retentates of the in vitro digests of phytate, there were no differences among the iron compounds ($P > 0.05$). In these digests, oxidation was lower than that in in vitro digests of water for all iron compounds ($P < 0.05$). Higher oxidative activity, different for the selected iron compounds, was observed in the dialyzates and the retentates of the in vitro digests of phytate solutions that were treated with 1.25 mg/mL or 0.625 mg/mL phytase before digestion ($P < 0.05$). This increase was not different for the two phytase levels ($P > 0.05$), although they represent 55.2% and 79.9% hydrolysis of the phosphate bonds, respectively.

Statistically significant differences among the selected iron compounds were observed. In the dialyzates of in vitro digests of water, NaFeEDTA and $FeCl_3$ had the lowest oxidative activity followed by ferrous lactate and ferrous bis-glycinate (Fig. 1). In the retentates of in vitro digests of water, ferrous lactate and $FeCl_3$ were the least active in oxidizing linoleic acid followed by NaFeEDTA and ferrous bis-glycinate (Fig. 1). In in vitro digests of phytate solutions treated with phytase, in both the dialyzate and the retentate, $FeCl_3$ was the least active, followed by ferrous lactate, ferrous bis-glycinate and NaFeEDTA (Fig. 1).

The percentage of dialyzable and soluble ferrous and total iron of the iron compounds added in in vitro digests of water or in phytate solution in the presence or absence of phytase is presented in Figs. 2 and 3. The percentage of ferrous dialyzable iron in in vitro digests of water was highest for the treatments that contained ferrous lactate ($P < 0.05$) (Fig. 2). In in vitro digests of phytate solutions, the percentage of ferrous dialyzable iron was lower than that in in vitro digests of water solutions for all iron

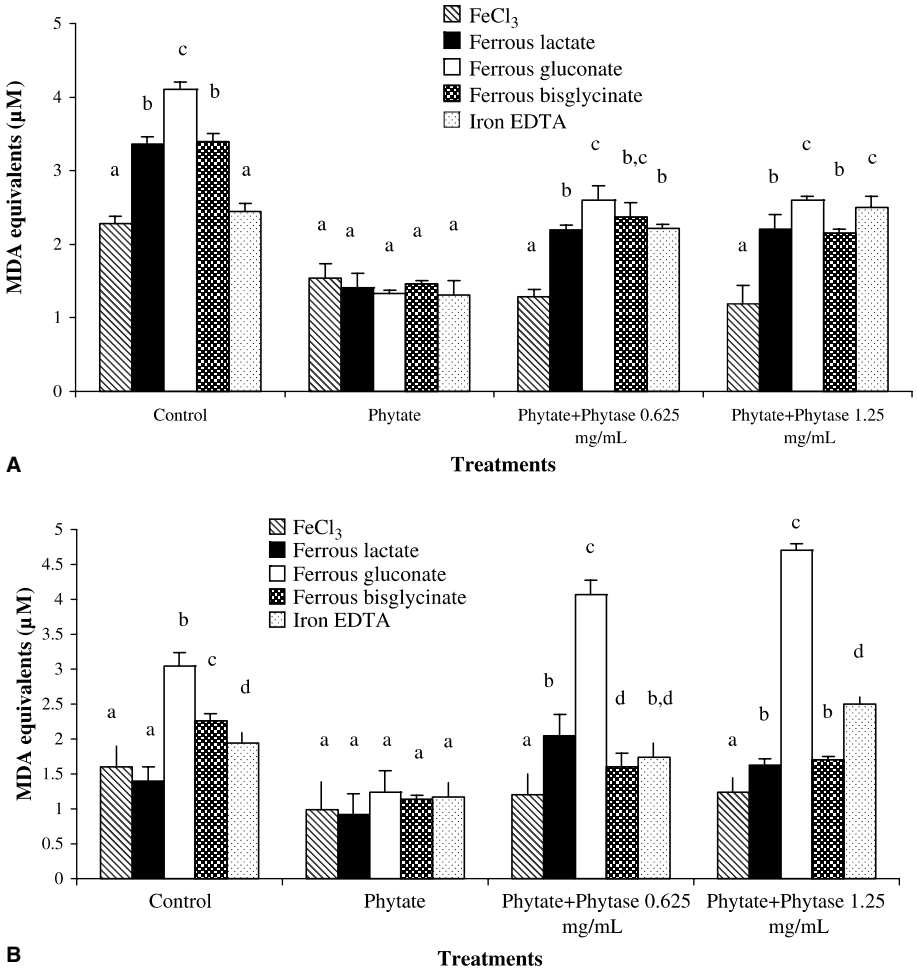

Fig. 1. Oxidation in mixtures of linoleic acid micelles and non-dialyzable (graph A) or dialyzable digests (graph B) of iron compounds added in water (control) or in phytate solutions in the presence or in the absence of phytase. Results are expressed as malonylaldehyde (MDA) equivalents and are means of three experiments. Means with different letters are significantly different ($P < 0.05$).

compounds ($P < 0.05$) (Fig. 2). Ferrous gluconate had the highest percentage of ferrous dialyzable iron in phytate digests ($P < 0.05$). In the presence of phytase, an increase in the percentage of dialyzable ferrous iron was observed in all treatments (Fig. 2). NaFeEDTA was the highest ($P < 0.05$) at in vitro digests of phytate treated with phytase at both levels. The percentage of ferrous dialyzable iron was similar at both phytase levels with the exception of iron bis-glycinate, which was lower at the higher phytase level (Fig. 2). Similar observations are reported for the concentration of ferrous dialyzable iron (Table 1).

The percentage of total dialyzable iron in all treatments followed similar trends with those observed in the formation of ferrous dialyzable iron when phytate or phytate and phytase were present (Fig. 2). Total dialyzable iron was highest in water digests of ferrous bis-glycinate ($P < 0.05$). In phytate digests, the percentage of total dialyzable iron was highest for NaFeEDTA and ferrous gluconate. NaFeEDTA was the highest ($P > 0.05$) at both phytase levels, followed by ferrous gluconate and ferrous lactate. Similar observations are reported for the concentration of total dialyzable iron (Table 1).

Percent soluble ferrous iron or total iron may be calculated from dialyzable and non-dialyzable iron (Kapsokefalou et al., 2005). Therefore, discrepancies are expected between reported in the comparisons for percent soluble ferrous or total iron and non-dialyzable ferrous or total iron (Figs. 3 and 4, Table 1).

Percent soluble ferrous iron (Fig. 3) in in vitro digests of water solutions of $FeCl_3$, ferrous lactate and NaFeEDTA was higher ($P < 0.05$) than that of ferrous gluconate or ferrous bis-glycinate. In in vitro digests of phytate solutions, the percentage of ferrous soluble iron was highest for NaFeEDTA, followed by ferrous gluconate and ferrous lactate. In the presence of phytase an increase in the formation of ferrous soluble iron was observed. NaFeEDTA was the highest ($P < 0.05$) at both phytase levels, followed by ferrous gluconate, ferrous bis-glycinate and ferrous lactate. The percentage of ferrous soluble iron was similar at both phytase levels ($P > 0.05$). Similar observations are reported for the concentration of ferrous non-dialyzable iron (Table 1).

Percent soluble total iron (Fig. 3) in in vitro digests of water solutions of ferrous gluconate was higher ($P < 0.05$)

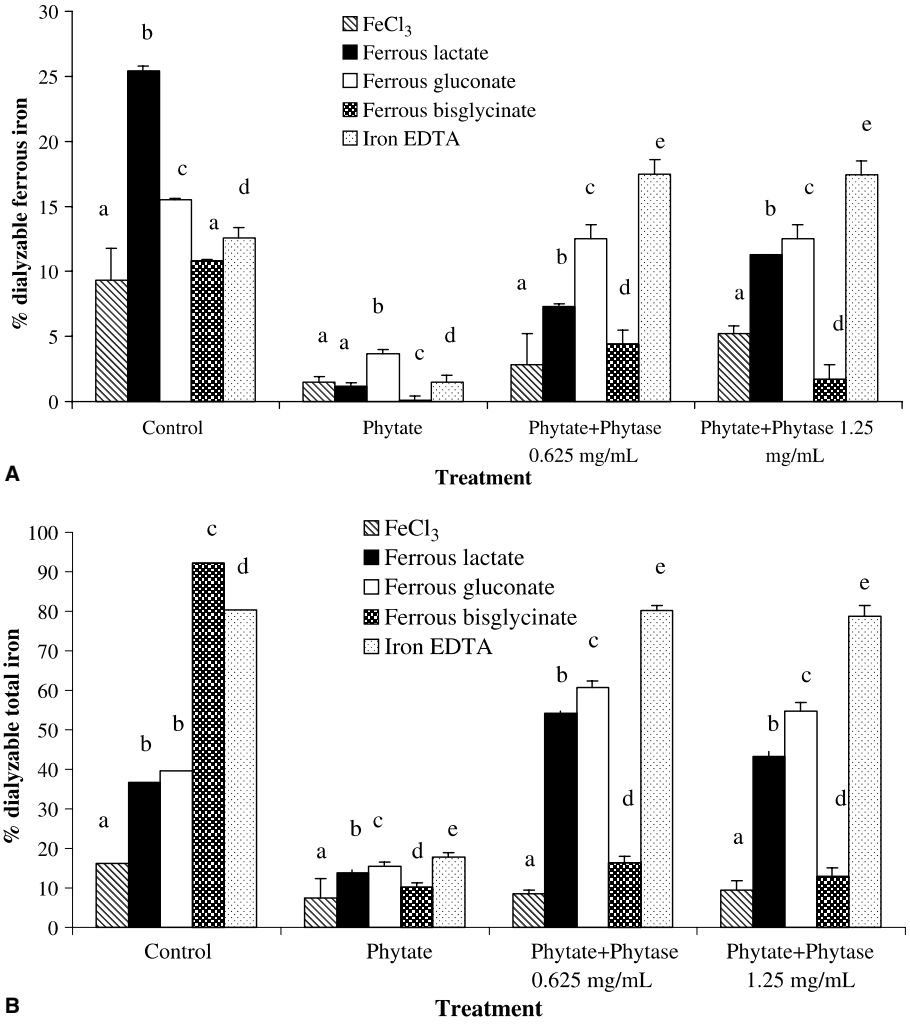

Fig. 2. Dialyzability (dialyzable ferrous and dialyzable total iron, graphs A and B, respectively) of selected iron compounds in water (control) or in phytate solutions digested in vitro in the presence or in the absence of phytase. Results are expressed as percentage of iron before incubation and are means of three experiments. Means with different letters are significantly different ($P < 0.05$).

than that of ferrous lactate, ferrous bis-glycinate or NaFeEDTA. In in vitro digests of phytate solutions, the percentage of soluble total iron was lower than that in water solutions. In in vitro digests of phytate NaFeEDTA was the highest in the percentage of soluble total iron. In the presence of phytase an increase in the percentage of soluble total iron was observed. NaFeEDTA was the highest ($P > 0.05$) at both phytase levels, followed by ferrous bisglycinate, ferrous gluconate and ferrous lactate. The percentage of soluble total iron was similar at both phytase levels. Similar observations are reported for the concentration of total non-dialyzable iron (Table 1).

The concentration of ferrous and total iron in the dialyzable and the non-dialyzable digests was correlated with oxidative activity (Figs. 4 and 5). The correlation coefficient (r^2) ranged from 0.2037 to 0.2838. For the dialyzable digests, the correlation coefficient (r^2) was 0.2037 and 0.2838 for ferrous and for total iron, respectively, while for the non-dialyzable digests, the r^2 was 0.2527 and 0.2626 for ferrous and total iron, respectively.

4. Discussion

In this study, the oxidative properties of selected iron compounds were correlated with dialyzability and solubility in in vitro digests of water or phytate solutions or phytate solutions treated with phytase.

The first finding reported herein is that the selected iron compounds studied exhibited variable oxidative activity under conditions that simulate the gastrointestinal environment. This refers to iron compounds employed in the fortification of foods. Therefore, it may reflect differences in potential oxidative properties. New developments in the study of oxidation processes in vivo have implied that iron that is not absorbed may catalyse oxidation reactions linked with adverse health effects (Lund et al., 2001).

The oxidative activity of iron has been studied before under in vitro conditions, particularly in in vitro digests (Matsingou, Kapsokefalou, & Salifoglou, 2000, 2001). However, in this work, a series of selected iron compounds employed in food fortification were perused, in an attempt

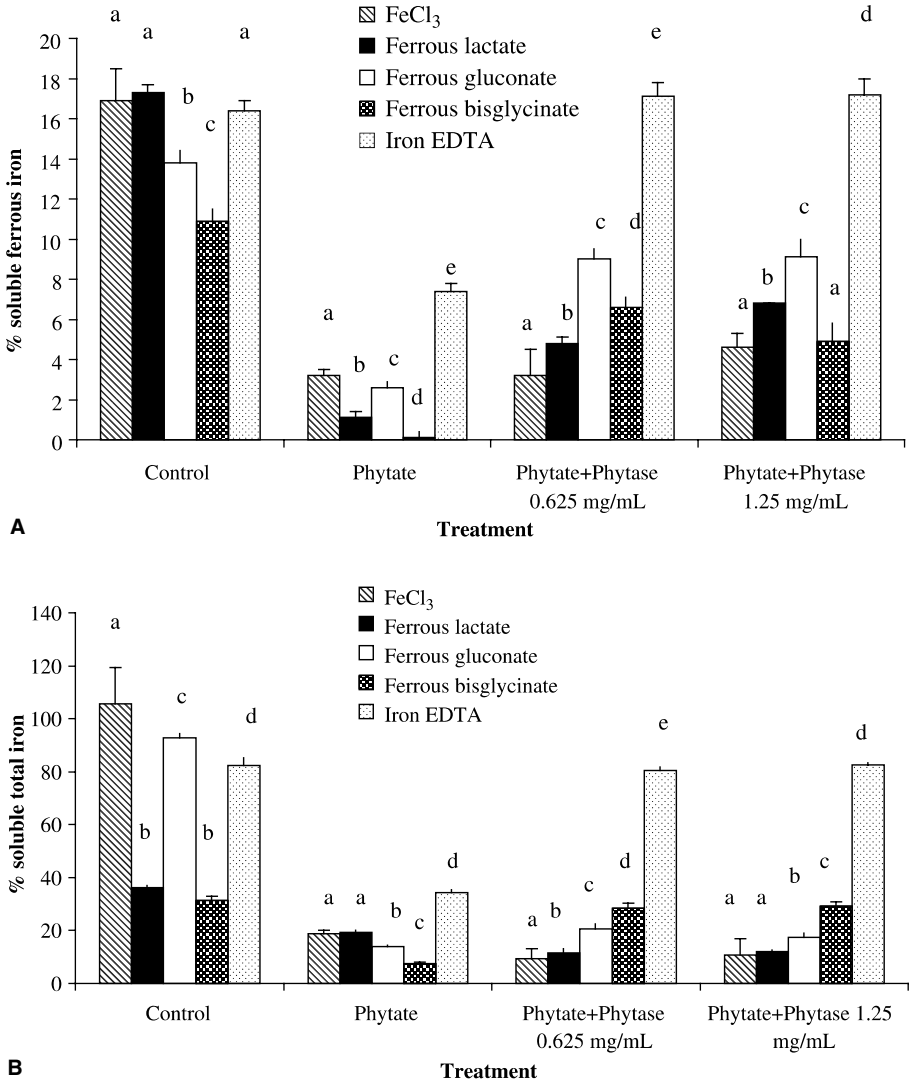

Fig. 3. Solubility (soluble ferrous and total iron, graphs A and B, respectively) of selected iron compounds in water (control) or in phytate solutions digested in vitro in the presence or in the absence of phytase. Results are expressed as percentage of iron before incubation and are means of three experiments. Means with different letters are significantly different ($P < 0.05$).

to seek and delineate their prooxidant activity in in vitro digests. Interest may be directed towards the retentate of the digests which is the non-dialyzable fraction that contains iron compounds that are insoluble and/or have MW higher than 6000. The retentate has been associated with the non-absorbable fraction that remains in the gastrointestinal lumen (Kapsokefalou & Miller, 1994). In the retentate of the in vitro digests, the selected iron compounds exerted different oxidative activity.

Differences in the oxidative activity of the selected iron compounds were expected because of the variable iron solubility and dialyzability observed in the in vitro digests (Figs. 2 and 3). Iron, promotes oxidation in the form of Fe^{2+} or of Fe^{3+} (Aruoma, Halliwell, Laughton, Quinlan, & Gutteridge, 1989), but non-ionic forms of iron are probably not able to catalyze these reactions (Jovanovic, Simic, Steenken, & Hara, 1998; Kapsokefalou & Miller, 2001; Khokhar & Owusu Apenten, 2003). Consequently, it may be suggested that the physicochemical form of iron may determine the catalytic activity of iron in oxidation reactions occurring in the gastrointestinal environment. To this respect, it was expected that differences would be observed in the oxidative properties of the dialyzates and the retentates because the provide iron with different physicochemical characteristics, including molecular weight, valence and concentration (Table 1). It appears, however, that solubility and dialyzability did not correlate with the oxidative effect of iron (Figs. 4 and 5). Some iron compounds, particularly iron gluconate, exhibit a distinct behavior of oxidant activity within the examined group of iron compounds that may not be explained on the basis of iron solubility or dialyzability. This may suggest that other factors, such as the stability of iron chelates, may be important determinants of the catalytic activity of iron in the in vitro digests.

The second finding reported herein is that all iron compounds responded to the antioxidant effect of phytate in a

Table 1
Concentration of dialyzable (ferrous and total) and non-dialyzable (ferrous and total) iron formed in samples of selected iron compounds in water (control) or in phytate solutions digested in vitro in the presence or in the absence of phytase

Treatment	Iron compound	Dialyzable ferrous iron (ppm)[A]	Dialyzable total iron (ppm)[A]	Non-dialyzable ferrous iron (ppm)[A]	Non-dialyzable total iron (ppm)[A]
Control	$FeCl_3$	0.47 ± 0.13^{d}	0.84 ± 0.25^{d}	1.26 ± 0.17^{a}	5.55 ± 0.71^{a}
	Ferrous lactate	1.33 ± 0.02^{a}	1.93 ± 0.05^{c}	0.50 ± 0.01^{d}	1.89 ± 0.07^{b}
	Ferrous gluconate	0.81 ± 0.13^{b}	2.05 ± 0.12^{b}	0.64 ± 0.04^{c}	4.69 ± 0.19^{a}
	Ferrous bis-glycinate	0.57 ± 0.03^{c}	4.84 ± 0.22^{a}	$0.57 \pm 0.05^{c,d}$	1.64 ± 0.32^{b}
	Iron EDTA	0.69 ± 0.02^{c}	4.40 ± 0.05^{a}	0.86 ± 0.01^{b}	4.30 ± 0.07^{a}
Phytate	$FeCl_3$	0.06 ± 0.02^{c}	0.39 ± 0.03^{d}	0.26 ± 0.03^{a}	0.98 ± 0.05^{b}
	Ferrous lactate	0.06 ± 0.01^{c}	0.72 ± 0.02^{b}	0.06 ± 0.01^{c}	1.02 ± 0.02^{a}
	Ferrous gluconate	0.19 ± 0.01^{a}	0.81 ± 0.07^{b}	0.09 ± 0.03^{c}	0.73 ± 0.08^{c}
	Ferrous bis-glycinate	0.05 ± 0.03^{c}	0.53 ± 0.07^{c}	0.08 ± 0.06^{c}	0.39 ± 0.04^{d}
	Iron EDTA	0.15 ± 0.01^{b}	1.80 ± 0.02^{a}	0.19 ± 0.01^{b}	0.90 ± 0.02^{b}
Phytate ± phytase 0.625 mg/mL	$FeCl_3$	0.15 ± 0.01^{d}	0.45 ± 0.06^{e}	0.18 ± 0.07^{d}	0.49 ± 0.09^{d}
	Ferrous lactate	0.38 ± 0.01^{c}	2.84 ± 0.08^{c}	0.13 ± 0.02^{d}	0.61 ± 0.03^{d}
	Ferrous gluconate	0.66 ± 0.05^{b}	3.18 ± 0.11^{b}	0.30 ± 0.04^{c}	1.08 ± 0.09^{c}
	Ferrous bis-glycinate	0.23 ± 0.05^{d}	0.86 ± 0.09^{d}	0.45 ± 0.06^{b}	1.49 ± 0.08^{b}
	Iron EDTA	0.92 ± 0.01^{a}	4.20 ± 0.08^{a}	0.89 ± 0.02^{a}	4.20 ± 0.03^{a}
Phytate ± phytase 1.25 mg/mL	$FeCl_3$	0.27 ± 0.04^{c}	0.49 ± 0.06^{d}	0.21 ± 0.02^{d}	0.55 ± 0.15^{d}
	Ferrous lactate	0.59 ± 0.02^{b}	2.27 ± 0.05^{b}	0.14 ± 0.03^{e}	0.63 ± 0.04^{d}
	Ferrous gluconate	0.65 ± 0.05^{b}	2.87 ± 0.15^{b}	0.31 ± 0.03^{c}	0.92 ± 0.06^{c}
	Ferrous bis-glycinate	0.09 ± 0.06^{d}	0.68 ± 0.10^{c}	0.40 ± 0.03^{b}	1.53 ± 0.03^{b}
	Iron EDTA	0.93 ± 0.02^{a}	4.20 ± 0.05^{a}	0.85 ± 0.03^{a}	4.10 ± 0.04^{a}

[A] Means ± standard deviation for three experiments. Values with different letters within a column are significantly different: $P < 0.05$.

similar manner, implying that the potential oxidative properties of iron compounds may depend on the presence of iron chelators derived from food. The antioxidant effect of phytate was lost when the phytate solutions were treated with phytase (Fig. 1). The antioxidant effect of phytate, demonstrated herein, has been discussed before in different experimental conditions (Graf, Empson, & Eaton, 1987; Lund et al., 2001). Midorikawa, Murata, Oikawa, Hiraku,

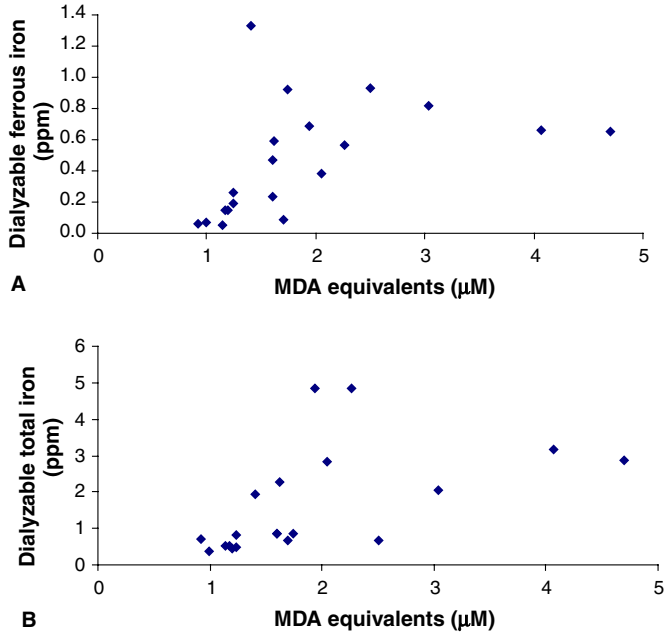

Fig. 4. Correlation of dialyzable ferrous and total iron concentration (graphs A and B, respectively) and oxidation, expressed as MDA equivalents, in mixtures of linoleic acid micelles and dialyzable digests of iron compounds added in phytate solutions in the presence or in the absence of phytase. The correlation coefficient was 0.2037 and 0.2838 for correlations presented in graphs A and B, respectively.

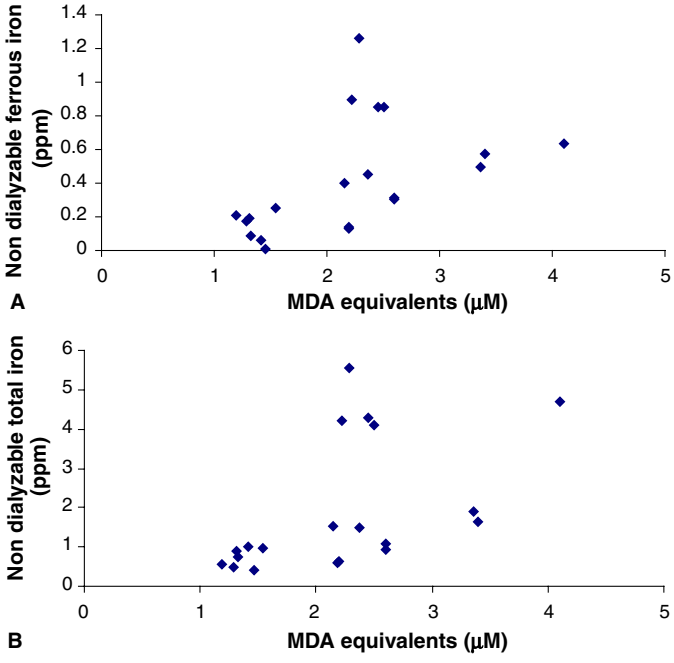

Fig. 5. Correlation of non-dialyzable ferrous and total iron concentration (graphs A and B, respectively) and oxidation, expressed as MDA equivalents, in mixtures of linoleic acid micelles and dialyzable digests of iron compounds added in phytate solutions in the presence or in the absence of phytase. The correlation coefficient was 0.2527 and 0.2626 for correlations presented in graphs A and B, respectively.

and Kawanishi (2001) concluded that phytic acid acts as an antioxidant to inhibit the generation of reactive oxygen species from H_2O_2 by chelating metals. Rimbach and Pallauf (1998) showed the antioxidant properties of phytic acid under in vitro conditions against ferrous sulfate. However, neither phytic acid nor iron, provided as ferrous sulfate, had any significant effect on liver oxidant or antioxidant status in vivo in growing rats. Moreover, in the intestinal environment of rats, particularly in colon, intrinsic phytate has produced lower levels of oxidation products when ferrous sulfate was the iron compound offered (Porres et al., 1999). These findings on the effect of phytate on ferrous sulfate are in agreement with those reported herein on other compounds that provide iron in the ferrous or in the ferric form. Miyamoto, Kuwata, Imai, Nagao, and Terao (2000) suggested that not only phytate but also intermediate hydrolysis products of phytate exhibit similar antioxidant properties, because they may chelate iron. This was also observed in the present study, where similar amounts of soluble iron and similar antioxidative properties were observed in phytate solutions treated with increased levels of phytase.

The third finding reported herein is that, the series of the selected iron compounds were compared and shown to exhibit variable solubility and dialyzability in water or phytate solutions or in phytate solutions treated with phytase. All iron compounds exhibited low ferrous and total iron solubility and dialyzability in in vitro digests of phytate solutions compared to in vitro digests of water solutions, which increased when the phytate solutions were treated with phytase. Ferrous gluconate and NaFeEDTA exhibited higher ferrous and total iron dialyzability than ferrous lactate or ferrous bis-glycinate in phytate solutions or in phytate solutions treated with phytase at both levels.

The significance of this finding lies in the association of the concentration of dialyzable ferrous iron with the prediction of iron bioavailability for this series of the selected iron compounds in phytate solutions or in phytate solutions treated with phytase. Clearly, the terms solubility and dialyzability are not synonymous to bioavailability. However, the in vitro method employed herein has been evaluated as a screening technique (Forbes et al., 1989; Hazell & Johnson, 1987) and may provide information when human studies are difficult to perform. Ferrous dialyzable iron has been proposed as a preferable index to total dialyzable iron (Miller, Schricker, Rasmussen, & Van Campen, 1981; Schricker, Miller, Rasmussen, & Van Campen, 1981), because it exhibits better correlation with data on iron absorption by humans (Kapsokefalou & Miller, 1991) or on iron uptake by cells (Glahn, Wien, Van Campen, & Miller, 1996). Although solely a predictor and not a true measurement of iron bioavailability, the concentration of ferrous dialyzable iron in in vitro digests relates to iron bioavailability because it depicts the affinity and interaction of iron with various dietary factors and their digestion products under conditions that mimic digestion. Solubility of ferrous or total iron is a prerequisite for any chemical reactivity of iron, nevertheless solubility is a less reliable index of iron bioavailability. Thus from the results reported herein, it may be predicted that ferrous gluconate and NaFeEDTA may exhibit higher bioavailability in phytate solutions or in phytate solutions treated with phytase, compared to ferrous lactate and ferrous bis-glycinate.

The inhibiting effect of phytate on iron bioavailability has been observed in vivo, in studies employing foods that contain phytate, mainly cereal foods, fortified with iron (Fox, Eagles, & Fairweather-Tait, 1998; Sandberg et al., 1999; Skoglund, Lonnerdal, & Sandberg, 1999). Phytate hydrolysis by phytase has been suggested as an acceptable means of decreasing phytate concentration is cereal foods and of increasing iron bioavailability (Hurrell et al., 2003; Sandberg, 2002; Stahl, Han, Roneker, House, & Lei, 1999). Skoglund et al. (1999) observed that the effect of phytate hydrolysis on iron solubilization was not linear. This may explain the finding that oxidative activity of the various iron compounds was similar in the presence of increased levels of phytase. Fox et al. (1998), compared iron bioavailability from infant cereals containing phytate fortified with iron glycine or ferrous sulfate and found no significant difference between these two iron compounds. However, Hurrell, Reddy, Burri, and Cook (2000) showed that iron absorption may be higher from cereal foods fortified with NaFeEDTA than when fortified with ferrous sulfate or ferrous fumarate. This suggests that, in the foods that contain phytate, the bioavailability of the various iron compounds varies because chelation of iron protects it from interactions with digestive components, allowing iron to remain soluble and enhancing its absorption (Hurrell et al., 2000). Therefore, the choice of the iron compound to be used in fortification is important.

In conclusion, ferric chloride, ferrous gluconate, NaFeEDTA, ferrous lactate and ferrous bis-glycinate exhibit variable oxidative activity, solubility and dialyzability in water or phytate solutions or in phytate solutions treated with phytase under conditions that simulate the gastrointestinal environment. The oxidative effect of iron did not correlate with solubility and dialyzability. This suggests that under the conditions of this experiment, the oxidative activity is not determined solely by the amount of iron present in solution. Other factors, such as presence of chelators derived from the iron compound itself or from the digest may be important. Clearly conclusions from in vitro studies present limitations and are difficult to interpret in humans. However, the results from this study encourage further investigation of the variable oxidative effect that iron compounds may exert in the gastrointestinal lumen. This may lead to the consideration of the oxidative potential of an iron compound as an important attribute in the selection for fortification, in addition to its bioavailability.

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