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In vitro estimation of iron and zinc dialysability from vegetables and composite dishes commonly consumed in Italy: effect of red wine

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Abstract

Selected vegetables (artichoke, asparagus, broccoli, cauliflower, cabbage, kale, carrot, potato), and some composite dishes (macaroni with vegetables) were analysed for their in vitro iron and zinc dialysability. The effect of red wine on iron dialysability from broccoli, potatoes and their respective composite dishes was also studied. Iron dialysability ranged from 10.7 to 23%, artichokes and asparagus showed lower values (5.7 and 7.7%, respectively). Zinc dialysability ranged from 19.5 to 49.3%. The generally high iron dialysability might be ascribed to organic acids (ascorbic, citric, malic acid). The addition of macaroni to vegetables drastically lowered both iron and zinc dialysability (from 40 to 75%). This effect was dependent on phytate content of macaroni (about 192 mg per dish). A negative relationship between red wine and iron dialysability was found. The decrease in iron dialysability was 45% in broccoli and 70% in macaroni with broccoli, 25% in potatoes and 46% in macaroni with potatoes. Present findings indicate that vegetables may contribute to get a useful level of absorbable iron and zinc in the diet.

Keywords: Iron; Zinc; Dialysability; Red wine; Vegetables; Composite dishes

1. Introduction

Non-heme iron represents the largest fraction of dietary iron. Vegetable intake in the average Italian diet is high (about 280 g/person/day) providing about 20% of the total dietary iron (unpublished data). Non-heme iron has, however, a low level of absorbability, being less effectively absorbed than heme iron (about 1–8% compared to 15–25% of heme iron). Vegetables contribute only 10% of the intake of zinc in the average Italian diet, meat being the most important source. Most of the studies on iron and zinc availability from plant foods deal with seeds (cereals, legumes) (Lombardi-Boccia, De Santis, Di Lullo & Carnovale, 1995; Lynch, Beard, Dassenko & Cook, 1984; Sandstrom, Almgren, Kivisto & Cederblad, 1987 1989). Few studies provide data on iron and zinc availability from vegetables. The absorption of both iron and zinc from vegetables can vary greatly because they are subject to the action of several food components exerting a powerful influence on their availability. Two constituents of vegetables, well known for their influence on iron availability, are organic acids and polyphenols. Ascorbic acid has long been recognized to enhance iron availability (Gillooly et al., 1983; Hazell & Johnson, 1987); the promoting effect of ascorbic acid on iron availability is dose-dependent and, moreover, it is effective in counteracting the action of naturally-occurring ligands such as phytate or polyphenols (Lombardi-Boccia, Di Lullo & Carnovale, 1991; Siegenberg et al., 1991). Because of the close relationship between the amount of ascorbic acid in foods or meals and the iron absorption increase, ascorbic acid is considered the major determinant of iron availability from the diet (Monsen et al., 1978). For other organic acids, a promoting action on iron absorption has also been reported. Studies have demonstrated the enhancing effect of citric acid on iron absorption; its effect has been shown to be additive to that of ascorbic acid (Ballot, Baynes & Bothwell, 1987; Hazell & Johnson). As far as zinc availability is concerned, no effect of ascorbic acid has been found (Solomons, Jacob, Pineda & Viteri, 1979), but a negative action of citrate has been demonstrated (Menard & Cousins, 1983; Turnbull, Blakeborough & Thompson, 1990). In contrast, polyphenols are a group of substances known to interfere with iron absorption. An example are the tannins in tea

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(Disler et al., 1975) and, as well, in other vegetable sources (Brune, Rossander & Hallberg, 1989; Gillooly et al.). The role of dietary fibre components on iron and zinc availability has not been completely clarified (Kelsay, Clark, Herbst & Prather, 1981; Sandstrom et al., 1987).

The present study has been undertaken in order to get a better knowledge of the nutritional significance of various vegetables in relation to iron and zinc nutriture. The estimation of iron and zinc availability from several vegetables was performed in vitro by using the method of Miller, Schricher, Rasreussen and van Campen (1981). Some of the selected vegetables are largely consumed in Italy, not only as vegetables, but also in composite dishes. Therefore, because the composition of the meal significantly influences non-heme iron and zinc availability, the effect of including vegetables in composite dishes prepared following traditional Italian recipes was studied. In addition, since red wine is usually consumed with meals, a relevant issue was also to study its effect on non-heme iron availability from foods or meals.

2. Material and methods

Vegetables (artichoke, asparagus, broccoli, cauliflower, cabbage, kale, carrot, potato), macaroni and red wine (Chianti, 1997; 12% alcohol by volume) were purchased locally.

All reagents were analytical grade and deionized water was used throughout. Glassware was acid-washed in concentrated HCl and rinsed with deionized water.

2.1. Cooking procedures

Non edible parts (leaves) of vegetables were removed; the vegetables were washed with deionized water and then cooked utilizing deionized water. Broccoli, cauliflower, cabbage and kale were pressure-cooked for 8 min in 150 ml of deionized water. Artichoke and asparagus were boiled. Potato and carrot were steamed. Tomatoes were analysed raw. Vegetables were freeze-dried before subsequent analysis. Composite dishes were prepared by boiling 80 g of macaroni in 1 l of deionized water with 2 g NaCl; macaroni was then strained and mixed with cooked vegetables (broccoli, cauliflowers artichoke, asparagus) (110 g, raw weight). Macaroni and potatoes were prepared by stewing 168 g of potatoes then 300 ml of deionized water were added, when boiling 80 g of macaroni were added and cooked for a further 10 min (the residual water was not discarded). Composite dishes were freeze-dried before analysis. For the experiments carried out with the addition of red wine, foods (broccoli and macaroni with broccoli, potato and macaroni with potato) were prepared as described above and then 130 ml of red wine (Chianti, 1997, 12% alcohol by volume) were added before freeze-drying the samples.

2.2. Iron and zinc analyses

These were performed by Atomic Absorption Spectrometry on a Varian SpectrAA 400 under standard conditions and following liquid ashing of the samples (4 ml $HNO_3 + 1$ ml H_2O_2) in a microwave digestion system. Wholemeal flour (BCR 189, Community Bureau of Reference, Brussels), was analysed as a check on the accuracy of the analysis. Experimental values were not statistically different from certified values (Fe: 69.3 ± 2.3 and 68.3 ± 1.9 mg/g, respectively; Zn: 56.2 ± 1.5 mg/g and 56.5 ± 1.7 mg/g, respectively).

2.3. Organic acids

Analyses were performed by HPLC. Samples were extracted in 5% HPO_3 (1:5, w:v) and then centrifuged at 2000 rpm. The supernatant was filtered through a 0.45 μm filter (Millipore Corporation, Bedford, MA) and 20 μl injected into an HPLC system. A Waters instrument equipped with a 510 pump model and a photodiode array detector (PAD), connected to a Millennium 2010, was used. Citric and malic acids were measured at 220 nm, ascorbic acid at 254 nm. Separation was performed with a 5 μm Hypersil ODS C18 column (250×4.5 mm, Sigma Aldrich, USA). The HPLC solvent system was potassium dihydrogen orthophosphate buffer (0.015 M, pH 2.8). Flow rate was 0.8 ml/min. Identification and quantification were done by comparison of sample peaks with those of external standards.

2.4. Polyphenols

The total polyphenol content was estimated according to the method of Singleton, Orthofer and Lamuela-Ravento's (1999).

2.5. Non-starch polysaccharides

Total, soluble and insoluble NSP contents were determined in duplicate using the method of Englyst and Cummings (1988).

2.6. In vitro iron and zinc dialysability

This was assessed by using the in vitro method of Miller et al. (1981). Aliquots of each samples were blended with 0.1 N HCl, the pH was adjusted to 2.0 ± 0.05, and 5 ml of pepsin solution (16 g pepsin-Porcine stomach, Sigma Chemical Co, St Louis, MO in 100 ml 0.1N HCl) were added. The final volume of the homogenates was brought to 100 g by adding deionized water and the samples were incubated at 37°C for 2 h in a shaking water bath. Aliquots of the pepsin digests (20 g), were transferred into 100 ml beakers. Segments of dialysis sac (MW cut-off 6-8000 Spectrapor I, Spectrum Medical Industries Inc., Los Angeles), were pretreated with 1% EDTA solutions and rinsed until the dialysis sacs were free of EDTA. The dialysis sacs were filled with 0.5 N $NaHCO_3$ (in a volume tested in a previous trial to give a pH of 7.5) and deionized water to obtain a volume of 20 ml, placed in beakers, and incubated for 30 min. When the pH reached 5.0, 5 ml of a pancreatin-bile solution (0.8g pancreatin, porcine pancreas, Sigma Chemical Co, St Louis, MO and 5 g bile, porcine, Sigma Chemical Co, St Louis, MO in 200 ml 0.1N $NaHCO_3$) were added and the incubation continued for a further 2 h. The dialysates were weighed and iron was determined by bathophenanthroline (Miller et al., 1981). Zinc was determined by atomic absorption spectrometry. Mineral content of the dialysis bag was calculated as a percentage of the total.

3. Results and discussion

The iron and zinc contents of both vegetables and composite dishes are listed in Table 1. Both minerals were in the range generally found in these vegetables (Carnovale & Marletta, 1997; Holland, Unwin & Buss, 1991). Composite dishes had slightly higher amounts of both iron and zinc compared to vegetables. Vegetables retained half the amounts of ascorbic acid after cooking, except *brassicacee* in which pressure-cooking induced losses of ascorbic acid of about 70% (Table 1). Citric acid was generally in high amounts, especially in tomato (496 mg/100 g); *brassica* vegetables were the richest in malic acid (Table 1). The polyphenol content of the samples varied widely ranging from 6.7 to 34 mg/100 g, only artichoke showed a particularly high amount (499 mg/100 g) (Table 1). The amounts of both organic acids and polyphenols detected in the composite dishes were lower than in vegetables alone and strictly dependent on the vegetable ingredients (Table 1). Table 2 shows the non-starch polysaccharide content of the samples and

Table 2
Total, insoluble and soluble non-starch polysaccharides (NSP) in vegetables (cooked and raw) and in composite dishes (g/100 g f.w.)[a]

Food source	NSP		
	Total	Soluble	Insoluble
Vegetables			
Broccoli	2.3 ± 0.4	1.0 ± 0.1	1.3 ± 0.3
Cauliflower	2.4 ± 0.1	1.1 ± 0.4	1.3 ± 0.5
Cabbage, green	2.5 ± 0.8	1.0 ± 0.5	1.5 ± 0.2
Kale	2.3 ± 0.1	0.9 ± 0.2	1.4 ± 0.4
Artichoke	3.7 ± 0.2	2.2 ± 0.4	1.5 ± 0.1
Asparagus	1.8 ± 0.1	0.8 ± 0.3	1.0 ± 0.3
Potato	1.4 ± 0.1	0.8 ± 0.2	0.6 ± 0.1
Carrot	2.0 ± 0.6	1.0 ± 0.8	1.0 ± 0.3
Tomato (raw)	0.8 ± 0.4	0.3 ± 0.3	0.5 ± 0.2
Composite dishes			
Macaroni with broccoli	2.6 ± 0.1	1.4 ± 0.1	1.2 ± 0.1
Macaroni with cauliflower	2.6 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
Macaroni with artichoke	2.5 ± 0.1	1.4 ± 0.1	1.1 ± 0.1
Macaroni with asparagus	1.3 ± 0.3	0.6 ± 0.3	0.8 ± 0.1
Macaroni with potato	1.4 ± 0.2	0.7 ± 0.2	0.7 ± 0.2

[a] Determined on duplicate samples (mean ± S.D.).

Table 1
Iron, zinc, ascorbic acid, citric acid, malic acid and polyphenol contents in vegetables (cooked and raw) and in composite dishes (f.w.)[a,b]

Food source	Fe (mg/100 g)	Zn (mg/100 g)	Ascorbic acid (mg/100 g)	Citric acid (mg/100 g)	Malic acid (mg/100 g)	Tannic acid (mg/100 g)
Vegetables						
Broccoli	0.67 ± 0.10	0.35 ± 0.13	32.0 ± 0.9	94 ± 9	198 ± 11	33.5 ± 9.8
Cauliflower	0.85 ± 0.81	0.52 ± 0.31	14.0 ± 0.3	194 ± 6	252 ± 12	23.2 ± 3.5
Cabbage, green	0.71 ± 0.23	0.53 ± 0.12	13.5 ± 0.7	70 ± 11	303 ± 14	12.8 ± 2.5
Kale	0.56 ± 0.30	0.19 ± 0.20	13.0 ± 1.0	128 ± 5	113 ± 3	34.0 ± 9.4
Artichoke	0.34 ± 0.21	0.55 ± 0.10	5.5 ± 0.1	186 ± 2	90 ± 9	499 ± 24
Asparagus	0.95 ± 0.80	0.78 ± 0.11	12.0 ± 1.3	137 ± 2	33 ± 2	23.9 ± 3.3
Potato	0.46 ± 0.12	0.51 ± 0.15	5.0 ± 0.1	197 ± 3	43 ± 1	6.7 ± 2.1
Carrot	0.34 ± 0.81	0.31 ± 0.52	2.0 ± 0.2	tr	134 ± 4	15.3 ± 2.8
Tomato, raw	0.36 ± 0.20	0.15 ± 0.20	18.8 ± 0.4	496 ± 2	77 ± 2	29.7 ± 2.3
Macaroni						
Macaroni with broccoli	0.80 ± 0.24	0.61 ± 0.11	0.2 ± 0.1	50 ± 7	77 ± 2	18.8 ± 4.1
Macaroni with cauliflower	0.92 ± 0.32	0.66 ± 0.21	1.3 ± 0.2	134 ± 3	101 ± 7	18.3 ± 3.8
Macaroni with artichoke	0.56 ± 0.11	0.77 ± 0.21	0.8 ± 0.5	nd	47 ± 3	131 ± 20
Macaroni with asparagus	0.53 ± 0.11	0.61 ± 0.13	nd	nd	2 ± 0.5	10.2 ± 4.5
Macaroni with potato	0.57 ± 0.10	0.82 ± 0.31	1.2 ± 0.2	98 ± 9	30 ± 1	3.7 ± 1.3

[a] Each value represents the mean ± S.D. of three determinations.
[b] nd not detectable; tr trace.

its proportions in soluble and insoluble fractions. *Brassica* vegetables were similar in non-starchy polysaccharide content (about 2.4 g/100 g) and in the relative soluble (about 1 g/100 g) and insoluble (about 1.4 g/100 g) fractions. These amounts were in agreement with those found in a previous study dealing with calcium availability from *brassica* vegetables (Lucarini, Canali, Cappelloni, Lullo & Lombardi-Boccia, 1998). Among vegetables, artichoke had the highest content of non-starch, polysaccharides and, together with potato, had the soluble fraction higher than the insoluble one (Table 2). The non-starch, polysaccharide composition of the composite dishes reflected the contribution of macaroni.

The in vitro iron and zinc dialysability from vegetables and composite dishes is shown in Table 3. Most vegetables displayed values of iron dialysability above 10%: artichokes and asparagus showed the lowest values (5.7 and 7.7%, respectively), and cabbage the highest (23%). Previous in vitro (Hazell & Johnson, 1987) and in vivo (Gillooly et al., 1983) studies reported values of iron dialysability close to those found in this study for carrot, potato, tomato and cauliflower; in the in vivo study, slightly higher values were found only for cauliflower and cabbage. Zinc dialysability from the vegetables studied was particularly high; it varied from 30.5 to 49.3%, except artichoke and carrot which showed lower amounts (Table 3). Few literature data are available on zinc absorption from vegetables. Sandstrom, Almgren, Kivisto and Cederblad (1987) reported values of zinc absorption over 90% for potato, carrot and cabbage.

Organic acids in vegetables are potentially involved in influencing iron dialysability. These vegetable constituents could likely bind iron and, consequently, account for a potential positive effect on its availability. This effect can be more or less marked, depending on the processes to which foods have been subjected. In this study vegetables, containing high amounts of one or more organic acids even after cooking, would be expected to display a high iron dialysability. Actually, among vegetables, only artichoke and asparagus showed values of iron dialysability lower than 10%. Polyphenols are a powerful factor affecting iron availability from vegetables. In this study we failed to find a direct relationship between polyphenols in foods and iron dialysability; in artichoke only the presence of polyphenols, in so exceptionally high amounts, can reasonably account for the low percentage of the iron dialysability found. Therefore the generally high level of iron dialysability found in this study is probably explained by the organic acid content of vegetables which may have counteracted the potential negative effect of endogenous phenolic compounds. Apart from citric acid which was reported to affect zinc absorption negatively (Menard & Cousins, 1983; Turnbull et al., 1990), neither organic acids nor polyphenols have shown any effect on zinc absorption (Solomons et al., 1979). The level of non-starch polysaccharides was similar among vegetables and did not explain some differences found in mineral dialysability among samples.

Table 3
In vitro iron and zinc dialysability from vegetables (cooked and raw) and in composite dishes (fresh basis)[a]

Food source	Fe (%)	Zn (%)
Vegetables		
Broccoli	13.1 ± 1.1	49.0 ± 0.9
Cauliflower	12.6 ± 1.3	37.3 ± 0.1
Cabbage, green	23.1 ± 0.2	32.7 ± 0.4
Kale	15.0 ± 0.2	49.3 ± 0.5
Artichoke	5.7 ± 0.1	24.8 ± 0.2
Asparagus	7.7 ± 0.4	30.5 ± 0.7
Potato	13.6 ± 0.3	34.7 ± 0.6
Carrot	10.7 ± 0.3	19.5 ± 0.4
Tomato, raw	15.0 ± 0.1	45.3 ± 0.3
Composite dishes		
Pasta with broccoli	6.6 ± 0.5	13.3 ± 0.7
Pasta with Cauliflower	4.7 ± 0.7	18.3 ± 0.5
Pasta with artichoke	3.9 ± 0.5	10.4 ± 0.3
Pasta with asparagus	3.8 ± 0.8	12.6 ± 0.2
Pasta with potato	8.2 ± 0.1	22.8 ± 0.5

[a] Each value represents the mean ± S.D. of triplicates.

The addition of cereal products (macaroni) to vegetables drastically lowered both iron ($P < 0.001$) and zinc ($P < 0.001$) dialysabilities compared to the respective vegetable (generally from 40 to 75% less) (Table 3). These reductions in mineral dialysability were likely dependent on the presence of wheat constituents affecting iron and zinc dialysability. Phytate in macaroni was the only constituent, not present in the vegetables studied, able to drastically affect the availability of both iron and zinc markedly. Macaroni generally contains about 2.4 mg/g of phytate (Harland & Oberleas, 1987; Plaami & Kumpulainen, 1995); the strong negative effect of phytate on iron and zinc availability is well known (Hallberg, Brune & Rossander, 1989; Lombardi-Boccia et al., 1991; Lonnerdal, Sandberg, Sandstrom & Kunz, 1989).

The influence of red wine on in vitro iron availability was studied in broccoli and potato and in their respective composite dishes. Because the experiments were carried out on freeze-dried samples, the red wine was dealcoholized. The addition of red wine caused an increase in the polyphenol content of samples which was doubled in potato and in its composite dish. As shown in Fig. 1 the addition of red wine to both broccoli and potatoes caused a reduction iron dialysability of about 45 and of 25%, respectively. The decrease in iron dialysability detected in composite dishes was stronger than that found in vegetables (70% less in macaroni with broccoli ($P < 0.001$) and 46% less in macaroni with potatoes ($P < 0.001$)). The finding that the inhibition of

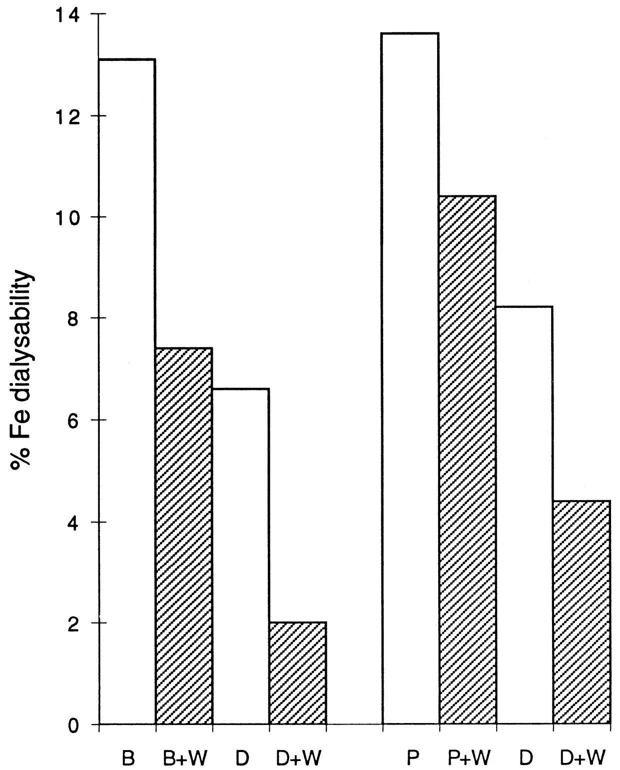

Fig. 1. Effect of the addition of red wine on iron dialysability from cooked broccoli and potato and their respective composite dishes (f.w.). Determined on triplicate samples. B, broccoli; B+W, broccoli +red wine; P, potatoes; P+W, potatoes +red wine; D, composite dishes; D+W, composite dishes +red wine.

iron dialysability doubled when vegetables were prepared together with cereals, suggests that the protein–phenols interaction, which makes protein less available for digestion (Baxter, Lilley & Williamson, 1997), may contribute to the impairment of iron availability by substracting the iron present in iron-containing peptides. Further studies are necessary to confirm this hypothesis. Bezwoda, Torrance, Bothwell, MacPhal, Graham and Mills (1985) in a human study found a significantly higher absorption of iron from white wine than from red wine and showed a significant increase in iron availability after removing 80% of polyphenols from red wine. Cook, Reddy and Hurrell (1995) comparing the iron absorption from a meal consumed with white wine and with two red wines they found a 3-fold higher absorption from white wine than from the red wine (highest in polyphenols).

The first finding of this study was the good level of dialysability of both iron and zinc displayed by the vegetables studied. This study furthermore emphasizes the particular importance of *brassica* vegetables in terms of mineral availability. Indeed, previous studies have already shown that *brassica* vegetables are a good source of available calcium (Heaney, Weaver, Hinders, Martin & Packard, 1993; Lucarini, Canali, Cappelloni, Di Lullo & Lombardi-Boccia, 1999). It is difficult to single out the relative effectiveness of each compound in influencing mineral availability, but presumably the generally high level of iron dialysability demonstrated in this study could mainly be ascribed to the high level of organic acids in these vegetables. As second finding of this study was the marked reduction in both iron and zinc dialysabilities subsequent to the inclusion of macaroni in the recipes. The addition of macaroni means an addition, calculated, of about 192 mg of phytate per dish which is, undoubtedly, the most powerful compound in terms of inhibition of iron and zinc absorption. Hallberg et al. (1989) reported that the addition of 100 mg of phytate to a standard meal reduced iron absorption by about 68%. A similar level of reduction in iron dialysability between vegetables and their respective composite dishes was found in this study. The same was found for zinc. Phytate in macaroni was thus able to overcome the positive influence of organic acids. A third finding of this study was the evidence of a clearcut relationship between red wine addition and impairment of iron availability. The 75% inhibition of iron absorption reported by Cook et al. (1995), in meals based on bread and red wine, was not far different from the reduction in iron dialysability found in the composite dishes (from 46 to 70%).

Non-heme iron absorption has a main role in diets like the Italian diet, in which non-heme iron is the predominant source of iron. Present findings indicate that vegetables may consistently contribute to a good level of absorbable iron and also zinc in the diet. Probably the question of the bioavailability of minerals could arise only when the intake of cereals is particularly high. Indeed, a great deal of evidence suggests that diets rich in vegetables do not necessarily tend to a low iron and zinc balance. Studies on humans have demonstrated that impaired iron and zinc nutriture in vegetarians is rarely detected despite the large content of factors known to inhibit their absorption (Anderson, Gibson & Sabry, 1981; Craig, 1994; Gibson, 1994).

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