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The Influence of Beverages and Condiments on *In Vitro* Estimated Iron Availability from Wheat Flour and Potato

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ABSTRACT

Beverages and condiments were mixed with either wheat flour or potato and were digested in vitro *under simulated physiological conditions. The proportion of iron diffusing across a semi-permeable membrane was used as an index of bioavailability. Tea, coffee, cocoa, milk, salad cream, tartare sauce and horseradish sauce greatly reduced iron diffusibility from potato ($P < 0{\cdot}001$). Lemonade, tonic water, Lucozade, orange juice, monosodium glutamate and salt greatly enhanced iron diffusibility from both potato and wheat flour ($P < 0{\cdot}001$). Certain constituents of the beverages and condiments are probably responsible for these effects. Therefore, the choice of beverage or condiment consumed with a meal may have a large influence on the availability of the iron in that meal.*

INTRODUCTION

Iron-deficiency is often caused by poor availability of dietary iron. Iron availability varies greatly between different foods depending on their content of inhibitors (e.g. phytic acid) or enhancers (e.g. ascorbic acid). One food will also influence iron availability from other foods consumed at the same time. For example, iron availability from plant foods is approximately doubled when consumed together with meat or orange juice (Layrisse *et al.*, 1968; Maisterrena *et al.*, 1977). Meat contains animal tissue protein and orange juice contains ascorbic and citric acids and these components have

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been shown to enhance iron availability (Cook & Monsen, 1976; Gillooly *et al.*, 1983). Conversely, iron availability is greatly reduced when eggs, tea, soya, or maize are included in the meal (Callender *et al.*, 1970; Disler *et al.*, 1975; Cook *et al.*, 1981; MacPhail *et al.*, 1981). Eggs contain phosvitin, tea contains tannins, and cereals and legumes contain phytic acid and fibre which have all been shown to inhibit iron availability (Sharpe *et al.*, 1950; Callender *et al.*, 1970; Fernandez & Phillips, 1982; Gillooly *et al.*, 1983).

Apart from the major food items a wide variety of beverages and condiments are also consumed with most meals. Relatively few studies have investigated their influence on iron availability. However, they often contain many natural and added ingredients capable of influencing the availability of dietary iron.

In the present study an *in vitro* technique was used to give an estimation of relative iron availability (Miller *et al.*, 1981). This method has been shown to give results consistent with those obtained in human absorption studies (Schricker *et al.*, 1981). Those interactions which influence iron availability *in vivo* have been shown to have the same influence in the *in vitro* method (Miller *et al.*, 1981). The present study was therefore designed to investigate the influence of beverages and condiments on *in vitro* estimated iron availability from two commonly consumed foods, wheat flour and potato.

MATERIALS AND METHODS

Preparation of food–beverage and food–condiment mixtures

Canned potatoes were drained and mashed, and plain white wheat flour was boiled for 15 min in distilled water. Each food was then thoroughly mixed with the appropriate quantity of the beverage or condiment being investigated and if necessary the mixture made up to 100 g with distilled water. According to McCance and Widdowson's Food Composition Tables plain white flour contains approximately 87% dry matter whereas canned potatoes contain approximately only 16% dry matter (Paul & Southgate, 1978). Therefore, either 10 g of white flour or 45 g of potato was added to each mixture so that, on a dry weight basis, each mixture contained approximately 7 to 9% white flour, or potato. Canned, rather than fresh, potatoes were used because the quality control applied to the selection and processing of canned potatoes ensured a more consistent product. Fresh potatoes are more variable (e.g. size, maturity) and this could lead to variations in iron availability (T. Hazell & I. T. Johnson, unpublished results).

The quantity of each beverage or condiment added to the white flour, or potato is shown in Tables 1 and 2. For each beverage or condiment the amount added to both potato and white flour is the same. The tea infusion was made using one tea bag immersed in 100 g of boiled tap water for 90 s. The appropriate quantities of dried cocoa powder or dried instant coffee granules (see Table 1) were dissolved in 50 g of boiled tap water. The appropriate quantity of concentrated Ribena (see Table 1) was diluted with 35 g of cold tap water. Therefore, 50 g infusions of tea and 50 g solutions of cocoa, coffee or Ribena were added to each food, and the mixtures made up to 100 g with distilled water.

Reagents

Sixteens grams of pepsin powder (from porcine stomach mucosa, Sigma Chemical Co., Poole, Dorset) were suspended in 0·1 N HCl and brought to 100 ml with 0·1 N HCl. Four grams of pancreatin from porcine pancreas (Sigma Chemical Co.), and 25 g of bile extract (porcine, Sigma Chemical Co.), were dispersed in 0·1 M $NaHCO_3$ and the mixture was brought to 1 l with 0·1 M $NaCHO_3$. Dialysis tubing was used with a molecular weight cut-off of approximately 12 000 (Medicell International Ltd, London). Radioactive ^{59}Fe as $FeCl_3$ in 0·1 N HCl was used (7·8 μg Fe/ml and 100 μCi/ml, Amersham International plc., Amersham, Bucks).

Analytical methods

The release of diffusible iron was determined according to the method of Miller *et al.* (1981). The pH of each food–beverage or food–condiment mixture was adjusted to 2·0 with 6 N HCl and the mixture was then spiked with ^{59}Fe. Three millilitres of the pepsin suspension were added to each mixture which was then incubated in a 37°C shaking water bath for 2 h. Duplicate 20 ml samples of the pepsin digest were then transferred to 125 ml plastic bottles. Segments of dialysis tubing containing 25 ml distilled water and an amount of $NaHCO_3$ equivalent to the titratable acidity measured previously (see below) were placed in each bottle. The bottles were incubated in a 37°C shaking water bath for 30 min. Five millilitres of pancreatin–bile extract mixture were added to each bottle and the incubation was continued for a further 2 h. At the end of the incubation period the dialysis tubes were removed and the contents of each bottle (weight of retentate) and dialysis tube (volume of diffusate) were measured and analyzed.

There is a complete physicochemical exchange between the trace amount of added radioactive iron and the total endogenous iron present in the food–condiment/beverage mixture (Monsen, 1974). Therefore the diffusion of added ^{59}Fe across the dialysis tubing represents the diffusion of total

TABLE 1
The Influence of Various Beverages on Fe Diffusibility from Wheat Flour and Potato[a]

White wheat flour (10 g)		*Percentage diffusible ^{59}Fe Mean*	*SE*	*Statistical significance of difference from control: P <*	*Potato (45 g)*		*Percentage diffusible ^{59}Fe Mean*	*SE*	*Statistical significance of difference from control: P <*
+ coffee granules	(2 g)	1·2	0·14	ns	+ tea infusion	(50 g)	1·5	0·03	0·001
					+ cocoa powder	(4 g)	3·5	0·21	0·001
Control[b]		1·5	0·17	—	+ coffee granules	(2 g)	4·3	0·24	0·001
					+ skimmed milk	(50 g)	6·0	0·68	0·001
+ tea infusion	(50 g)	1·9	0·24	ns	+ whole milk	(50 g)	7·8	1·20	0·001
+ beer	(50 g)	2·4	0·28	0·05	+ Ribena	(15 g)	11·8	0·63	ns
+ Coca Cola	(50 g)	2·4	0·15	0·05	+ red wine	(50 g)	12·1	0·91	ns
+ white wine	(50 g)	5·6	0·61	0·001	+ Coca Cola	(50 g)	12·4	0·66	ns
+ Ribena	(15 g)	8·7	0·53	0·001					
+ Lucozade	(50 g)	16·9	0·18	0·001	Control[b]		13·2	0·37	—
+ tonic water	(50 g)	17·9	1·12	0·001					
+ lemonade	(50 g)	23·6	1·20	0·001	+ white wine	(50 g)	14·7	1·02	ns
+ orange juice	(50 g)	25·0	0·50	0·001	+ beer	(50 g)	15·2	1·35	ns
					+ lemonade	(50 g)	18·6	0·56	0·001
					+ Lucozade	(50 g)	18·8	1·17	0·001
					+ tonic water	(50 g)	22·2	0·76	0·001
					+ orange juice	(50 g)	23·9	2·04	0·001

[a] Values are means of five observations.
[b] Control is food and distilled water.
ns, not significant.

TABLE 2
The Influence of Various Condiments on Fe Diffusibility from Wheat Flour and Potato[a]

White wheat flour (10 g)		*Percentage diffusible ^{59}Fe*		*Statistical significance of difference from control: P <*	*Potato (45 g)*		*Percentage diffusible ^{59}Fe*		*Statistical significance of difference from control: P <*
		Mean	*SE*				*Mean*	*SE*	
Control		1·5	0·17	—	+ salad cream	(35 g)	1·7	0·15	0·001
					+ tartare sauce	(35 g)	4·0	0·17	0·001
+ sugar	(5 g)	1·5	0·26	ns	+ horseradish sauce	(35 g)	7·1	0·53	0·001
+ MSG[c]	(1 g)	1·8	0·08	ns	+ apple sauce	(35 g)	12·1	0·56	ns
+ salt	(1 g)	3·1	0·38	0·005					
+ vinegar	(35 g)	4·0	0·34	0·001	Control[b]		13·2	0·37	—
+ pickle	(35 g)	4·9	0·95	0·001					
+ brown sauce	(35 g)	7·1	0·18	0·001	+ tomato ketchup	(35 g)	14·0	0·38	ns
+ salt	(5 g)	7·5	0·73	0·001	+ mint sauce	(35 g)	15·6	1·32	0·025
+ MSG[c]	(5 g)	8·3	0·51	0·001	+ vinegar	(35 g)	17·2	0·93	0·001
+ apple sauce	(35 g)	12·0	1·00	0·001	+ MSG[c]	(5 g)	18·3	1·20	0·001
+ tomato ketchup	(35 g)	14·0	0·94	0·001	+ salt	(5 g)	19·4	0·53	0·001
					+ salt and MSG[c]	(2·5 g) (2·5 g)	19·6	0·98	0·001

[a] Values are means of five observations.
[b] Control is food and distilled water.
[c] MSG is monosodium glutamate.
ns, not significant.

endogenous iron (Miller *et al.*, 1981). Radioactivity in the diffusates and retentates was determined by counting 5 ml or 5 g samples in a Philips automatic γ counter. The percentage of diffusible (dialysable) ^{59}Fe was determined using the following equation:

$$\frac{\text{cpm } ^{59}\text{Fe/ml diffusate} \times \text{ml diffusate}}{(\text{cpm } ^{59}\text{Fe/ml diffusate} \times \text{ml diffusate}) + (\text{cpm } ^{59}\text{Fe/g retentate} \times \text{g retentate})} \times 100\%$$

Measurement of ^{59}Fe by γ counting is simpler and quicker than the measurement of total iron by atomic absorption spectrophotometry. However, the diffusates and retentates of some samples were also analyzed for iron by atomic absorption spectrophotometry, and showed good agreement with results determined by measurement of ^{59}Fe. Miller *et al.* (1981) also found good agreement between radioactive and chemical measurements of percentage diffusible iron.

Titratable acidity was determined on 20 g samples of the pepsin digest to which 5 ml of the pancreatin–bile extract mixture had been added. Titratable acidity was defined as the number of equivalents of NaOH required to titrate the combined pepsin digest pancreatin–bile extract mixture to pH 7·0 (0·5 M NaOH was used in the titration).

RESULTS

The beverages and condiments tested were found to be capable of both enhancing and inhibiting *in vitro* estimated iron availability (i.e. Fe diffusibility). In Tables 1 and 2, the condiments and beverages listed above the control (control is food with distilled water) inhibited iron diffusibility whereas those listed below the control enhanced iron diffusibility.

The beverages can be divided into three groups according to their degree of influence on iron diffusibility (Table 1). Tea, cocoa, coffee and milk all greatly reduced iron diffusibility from potato ($P < 0{\cdot}001$), although tea and coffee did not influence iron diffusibility from white flour (Table 1). Although Coca Cola, Ribena, wine and beer did not affect iron diffusibility from potato, they did marginally but significantly enhance iron diffusibility from white flour ($P < 0{\cdot}05$). Lemonade, Lucozade, tonic water and orange juice all greaty enhanced iron diffusibility from both foods ($P \sim 0{\cdot}001$).

Salad cream, tartare sauce and horseradish sauce all greatly reduced iron diffusibility from potato ($P < 0{\cdot}001$) (Table 2). Salt (5 g), monosodium glutamate (5 g) and vinegar significantly enhanced iron diffusibility from both potato and white flour ($P < 0{\cdot}001$). Mint sauce, brown sauce and pickle also exerted an enhancing effect ($P < 0{\cdot}025$), but sugar (5 g) did not. Tomato ketchup and apple sauce greatly enhanced iron diffusibility from white flour ($P < 0.001$) but they had no effect on the iron in potato (Table 2).

DISCUSSION

Apart from studies involving orange juice or tea, relatively few investigations have been performed on the effects of different beverages on iron availability (Hallberg & Rossander, 1982; Morck *et al.*, 1983). The influence of condiments on iron availability is largely unknown.

The results in Table 1 are in qualitative agreement with those obtained in human absorption studies. Of all the beverages, orange juice and tea had the greatest influence on iron diffusibility. Tea reduced iron diffusibility from 13·2% to only 1·5% in potato, and orange juice enhanced iron diffusibility from 1·5% to 25·0% in white flour (Table 1). The effects of tea in reducing, and of orange juice in promoting, iron absorption from foods are well documented (Maisterrena *et al.*, 1973; Disler *et al.*, 1975). Coffee, cocoa and milk reduced iron diffusibility from potato in the present study (Table 1) and have also all been found to reduce iron availability in human absorption studies (Sharpe *et al.*, 1950; Rossander *et al.*, 1979; Morck *et al.*, 1983).

Certain components contained in the foods listed in Tables 1 and 2 are probably responsible for the observed effects on iron diffusibility. For example, tannin and chlorogenic acid readily form darkly coloured insoluble iron complexes which are unavailable for absorption (Disler *et al.*, 1975; Gray & Hughes, 1978; Gillooly *et al.*, 1983). Both are examples of polyphenols, a group of compounds abundantly present in tea, coffee and many other plant foods (Gray & Hughes, 1978; Somogyi, 1978; Narasinga Rao & Prabhavathi, 1982; Gillooly *et al.*, 1983). Phytic acid is another potent inhibitor of iron availability (Apte & Venkatachalam, 1962) and is present in numerous plant foods including cocoa (Oberleas, 1973; Paul & Southgate, 1978). Most of the iron in milk is bound to lactoferrin (Gurr, 1981). This compound has been found to depress iron availability (McMillan *et al.*, 1977) and is probably responsible for the reduction in iron diffusibility caused by the addition of milk to potato (Table 1).

The increase in iron diffusibility caused by the addition of orange juice is probably due to its large content of citric and ascorbic acids which are both naturally present in citrus fruits (Paul & Southgate, 1978). Citric and ascorbic acid have both been found to enhance iron availability whether measured *in vivo* (Gillooly *et al.*, 1983) or *in vitro* (Hazell & Johnson, 1987). Lemonade, Lucozade and tonic water also greatly enhanced iron diffusibility (Table 1). This is probably due to the large amounts of citric acid added during manufacture of this type of food product. However, other beverages were much less effective (Table 1). Coca Cola, Ribena, wine, and beer, although quite acidic, did not contain such high levels of citric acid. Coca Cola does contain large amounts of phosphoric acid and wine contains tartaric acid (Hartmann & Hillig, 1934) but these particular organic acids do not appear to be so effective in promoting iron availability.

The beverages listed in Table 1 also contain numerous other additives. These include glucose, sucrose, carbon dioxide, lactic acid, quinine, saccharin, sodium benzoate, sodium metabisulphite, caffeine, tartrazine and sunset yellow. The influence of these on mineral availability is mostly unknown.

Of the condiments listed in Table 2, salad cream, tartare sauce and horseradish sauce greatly depressed iron diffusibility but salt and monosodium glutamate were both powerful enhancers.

Salad cream, tartare sauce and horseradish sauce contain a wide range of ingredients. These include; vinegar, wine, egg yolk, salt, sugar, acetic acid, cream, lemon juice and vegetables. It seems probable that egg yolk is the ingredient responsible for the marked inhibitory influence of these products on iron diffusibility. Most of the other ingredients would be expected to enhance iron diffusibility and indeed the other sauces (i.e. mint, apple, pickle, brown and tomato) which contained similar ingredients but no egg yolk all displayed enhancement of iron diffusibility (Table 2). Egg yolk contains phosvitin, a powerful binder of iron which renders it insoluble and almost totally unavailable for absorption (Halkett *et al.*, 1958; Bjorn-Rasmussen *et al.*, 1972). Numerous *in vivo* studies have shown the detrimental influence of eggs and egg yolk on iron availability from other foods consumed at the same time (Schulz & Smith, 1958; Callender *et al.*, 1970; Cook & Monsen, 1976).

Brown sauce and tomato ketchup enhanced iron diffusibility from wheat flour (Table 1). Both contain large amounts of tomato which is a natural ingredient that contains highly available iron (Gillooly *et al.*, 1983; Hazell & Johnson, 1987). Rather surprisingly, tomato ketchup and apple sauce, which displayed the greatest enhancement of iron diffusibility from white flour, had no effect on potato (Table 2).

The sauces listed in Table 2 also contained numerous other additives. These included: guar gum, xanthan gum, locust bean gum, pectin, alginate, tartaric acid, lactic acid, sulphur dioxide, titanium dioxide and tartrazine. The influence of these on mineral availability is mostly unknown.

The greatest enhancement in both foods was caused by high levels of salt (i.e. 5 g) and monosodium glutamate (i.e. 5 g). Lower levels (i.e. 1 g) were less effective (Table 2). The influence of these two condiments is potentially very important because salt is widely consumed with most meals and both salt and monosodium glutamate are important ingredients in numerous processed food products. For example, snack foods may contain as much as 16% of such flavouring additives (Booth, 1984). Moreover, in areas of the world where iron-deficiency is widespread, salt has proved a very popular and successful vehicle for iron fortification (Narasinga Rao, 1985). The use of monosodium glutamate as a vehicle for iron fortification has also been considered (Garby, 1985). As well as the benefits of being inexpensive, widely consumed and not reacting adversely with the iron source, salt and monosodium glutamate may also enhance the availability of the fortification iron which they carry.

It should perhaps be pointed out that normal consumption of condiments would probably be much lower than the levels used to produce the effects displayed in Table 2. Therefore the results in Table 2 can only be a qualitative indication of what might be expected to occur following consumption of these condiments. Nevertheless, it is important to know the potential influence which these condiments could have if consumed in large amounts or in combination with other enhancers or inhibitors of iron availability. The beverages, on the other hand, were added in amounts which probably reflect normal consumption (see Table 1).

In conclusion, the present study shows that the choice of beverage or condiment consumed with a meal may have a large influence on the availability of the iron in that meal. This influence could be either beneficial or detrimental and is probably due to the presence of compounds which are known to act as inhibitors or promoters of iron absorption. However, these foods also contain many other compounds whose influence on iron availability in man has yet to be investigated.

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