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Determination of Iron in Foods by the Atomic Absorption Spectrophotometric and Colorimetric Methods

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ABSTRAK

Satu kajian perbandingan penentuan kandungan zat besi dalam pelbagai jenis makanan telah dijalankan dengan menggunakan kaedah penyerapan atom spektrofotometer (AAS) dan kaedah metri warna melalui tindak balas dengan fenantrolin. Sejumlah 156 jenis makanan, yang terdiri daripada 8 kumpulan makanan telah dikaji. Bagi setiap makanan (dianalisis secara duplikat), larutan abu telah disediakan dan satu alikuot telah dianalisis dengan kaedah AAS, manakala satu lagi dengan kaedah fenantrolin. Nilai min bagi analisis duplikat setiap makanan telah dibentangkan mengikut kumpulan makanan. Keputusan daripada kaedah AAS dan fenantrolin menunjukkan keselarian yang baik dengan koefisien korelasi 0.987. Analisis statistik dengan menggunakan ujian "berpasangan t" menunjukkan perbezaan keputusan dari kedua-dua kaedah adalah tidak bererti ($p < 0.05$) bagi 5 kumpulan makanan. Walaupun perbezaan yang bererti telah diperhatikan bagi 3 kumpulan makanan yang lain, angka statistik-t yang diperolehi hanya melebihi sedikit sahaja di atas paras bererti. Hasil bilas yang diperolehi bagi kedua-dua kaedah adalah memuaskan dan tidak mempunyai perbezaan yang bererti. Akan tetapi, varians bagi kaedah fenantrolin lebih tinggi sedikit. Hasil kajian menunjukkan bahawa kedua-dua kaedah tersebut dapat digunakan dengan memuaskan untuk analisis zat galian ini.

ABSTRACT

A comparative study of the determination of iron in a wide variety of foods was carried out using the atomic absorption spectrophotometric (AAS) and phenanthroline colorimetric methods. A total of 156 foods, belonging to 8 food groups were studied. For each food (determined in duplicate), ash solution was prepared and an aliquot subjected to AAS analysis, while another aliquot was determined by the phenanthroline method. Mean values for duplicate analysis of each food determined by the two methods were tabulated according to food groups. Results obtained by the AAS and phenanthroline methods showed good general agreement, with a correlation coefficient of 0.987. Statistical analysis using paired t-test showed that for 5 food groups, there was no significant difference ($p < 0.05$) in results given by the two methods. Although a significant difference was observed for the remaining 3 groups, the t-statistic calculated was just above the significance level. Recovery values given by the two methods were satisfactory, and were not significantly different. Variance for the phenanthroline method was, however, slightly higher. Results of the study suggest that both methods can be used satisfactorily for the analysis of this mineral.

INTRODUCTION

Iron deficiency anaemia has long been recognized, and is still an important nutritional deficiency problem in the country, afflicting particularly the vulnerable groups. (Tee, 1985). Thus, there has always been an interest in identifying local foods rich in iron.

Early methods for the determination of iron in foods had relied on the gravimetric procedure. Morris and Rosedale (1935) reported the precipitation of iron in foods with ammonium nitrosophenyl hydroxylamine ("cupferron"), followed by separation and weighing of the mineral as ferric oxide. Subsequently, there was a switch over to colorimetric procedures for the determination of this mineral. Simpson *et al.* (1951) estimated iron based on colour development with thioglycolic acid. Two years later, Leong (1953) reported the use of ortho-phenanthroline for colour development. This colour reagent, as well as bipyridyl, continued to be used by subsequent investigators (Tee *et al.* 1987). Determination of iron using atomic absorption spectrophotometry (AAS) has been introduced in recent years.

The choice of either the AAS or colorimetric method has relied on various factors, including availability of the required instrument as well as expertise. For various reasons, it would be important to determine if the AAS and colorimetric methods give comparable results. Different laboratories participating in a joint programme for the analysis of iron using the two different methods would need to determine if the results obtained are comparable. Before switching over to a newly purchased atomic absorption spectrophotometer, a laboratory would need to find out if the results to be obtained would be comparable to those previously obtained with the colorimetric method. On the other hand, in a laboratory using the AAS method, it may be necessary to switch to the colorimetric method if the spectrophotometer breaks down for a considerable length of time.

This report presents results of a comparative study of the determination of iron in a wide variety of foods using the AAS and colorimetric methods. It is hoped that the results would indicate clearly if significant differences are given by the two analytical procedures. This could be of assistance to laboratory workers intending to use either methods, such as in situations mentioned above. The study was carried out together with a comparative study of the determination of calcium using the AAS and titrimetric methods (Tee *et al.* 1989).

MATERIALS AND METHODS

Samples of foods from various food groups were purchased from local markets and retail stores for analysis. Wherever applicable, refuse in each food item was removed and its proportion in the food determined. The edible portions were blended and aliquots taken for analysis.

An amount of 5-15 g of the homogenized sample was dried in an air oven at 105°C for 3 hours. The dried sample was next charred until it ceased to smoke. The charred sample was then ashed in a muffle furnace at 550°C until a whitish or greyish ash was obtained. The ash was treated with concentrated hydrochloric acid, transfered to a volumetric flask and made up to 50 ml. For each food studied, two ash solutions were prepared, i.e. duplicate analysis was carried out. An aliquot of each ash solution was used for the determination of iron by the AAS method and another aliquot by the colorimetric method.

For the AAS method, a Varian Atomic Absorption Spectrophotometer model 175 with an air- acetylene flame was used. Wavelength was set to 248.3 nm for solutions with iron concentrations ranging from 2.5 to 10 μg/ml, or 327.0 nm for concentrations ranging from 25 to 100 μg/ml. Ferric nitrate solution for atomic absorption spectrophotometry (BDH) was used as standard. A calibration curve with at least 4 concentrations of iron within the analytical range was prepared. Concentrations of iron in test solutions was calculated from the standard curve prepared. For each ash solution, at least three readings were obtained and the average calculated.

In the colorimetric procedure, an aliquot of the ash solution was reacted with 1,10-phenanthroline hydrochloride and the resulting red complex read in a uv-vis spectrophotometer at 510 nm. For each ash solution, two tubes were prepared for reaction with the colour reagent and the average absorbance reading used for calcultion. A standard curve was prepared using ferric nitrate solution and used for calculation of iron in the test ash solutions. Analytical grade iron wire and ferrous ammonium sulphate have also been found to be suitable for use. The latter tend to be unstable and turn yellow on keeping.

Recovery studies were performed by adding a known amount (about 50% of the estimated iron content of the food) of iron stock standard to the food. Preparation of ash solution and analysis of iron using the AAS and colorimetric methods were carried out as described above.

Details of the AAS and colorimetric methods used are described in Tee *et al.* (1987). All results were expressed as per 100 g edible portion of the food. Mean values for duplicate analysis of each food determined by the two methods were calculated and results tabulated according to food groups. For each food group, the paired t-test was carried out using the ABSTAT statistical programme to determine if the two methods gave significantly different results. Correlation coefficient was calculated using the same programme. Analytical process standard deviations of the two methods were compared using the F-ratios method (Wernimont 1985).

RESULTS AND DISCUSSION

A wide variety of foods from various food groups were studied, to determine if the nature of the foods affected the results obtained. A total of 156 foods, belonging to 8 food groups were studied. Mean values for duplicate analysis of each food determined by the AAS and phenanthroline colorimetric methods were tabulated according to food groups (Tables 1 to 8). In all the tables, the English names of the foods are given, and arranged in alphabetical order. Where these names may be ambiguous or unclear, or when the English names are not known, the local names of the foods are included. The scientific names of the foods are also tabulated where appropriate.

There was generally good agreement in the results obtained by the two methods, as can be seen from Tables 1 to 8, and the scatter diagram plotting 150 pairs of results obtained (*Fig. 1*). The remaining 6 pairs were omitted from the plot as they were much higher than the majority of the values obtained. A good correlation coefficient of 0.987 was obtained for all 156 pairs of results.

Results of paired t-test for all food groups studied (Table 9) showed that for 5 food groups, there was statistically no significant difference ($p < 0.05$) in iron content determined by the AAS and colorimetric methods. For the remaining 3 groups, a significant difference in results was obtained. However, in all these cases, the t-statistic calculated was small, just above the significance level.

Determination of spiked iron in the foods was carried out in 10 separate studies. Table 10

TABLE 1
Iron in cereals and products as determined by the atomic absorption spectrophotometric and colorimetric methods

	mg Fe/100 g edible portion	
English/local name	AAS method	Colorimetric method
Bread, coconut	1.21	1.39
Bread, ryemeal	3.59	3.43
Bread, white	2.40	2.33
Bread, wholemeal	3.33	3.18
Noodle *laksa*, thick, dry	2.53	2.42
Noodle *laksa*, thick, wet	0.25	0.27
Oats, processed	2.66	4.16
Oats, rolled	2.01	3.49
Rice, broken	1.64	2.54
Rice bran, coarse	12.38	17.96
Rice noodle (*Loh-see-fun*)	0.28	0.39
Wheat flour, high protein	3.37	5.10
Wheat flour, wholemeal	4.10	8.14
Wheat germ	8.44	8.67

Each value is the mean of duplicate analysis

TABLE 2
Iron in legumes and products as determined by the atomic absorption spectrophotometric and colorimetric methods

	mg Fe/100 g edible portion	
	AAS method	Colorimetric method
Baked beans, canned	2.11	2.11
Chickpea/Common gram	4.99	5.14
Dhal, Mysore	5.46	5.85
Soya bean, fermented (*Tempeh*)	3.09	2.48
Soya bean cake (*Tau-kua*), spiced	5.32	4.46
Soya bean cake (*Tau-kua*)	2.27	2.53
Soya bean curd, sheets (*Fucok*)	7.06	8.23
Soya bean curd, strands (*Fucok*)	9.01	10.02
Soya bean curd (*Tau-hoo-fa*)	0.28	0.42
Soya bean curd (*Tau-hoo-pok*)	3.30	3.48
Soya bean milk, packet	0.17	0.23
Soya bean milk, unsweetened	0.34	0.34
Soya bean noodles	1.37	1.38

Each value is the mean of duplicate analysis

TABLE 3
Iron in nuts and seeds as determined by the atomic absorption spectrophotometric and colorimetric methods

		mg Fe/100 g edible portion	
English/local name	Scientific name	AAS method	Colorimetric method
Almond	*Prunus amygdalus*	3.00	3.34
Arecanut shavings	*Areca catechu*	5.44	7.39
Brazil nut	*Bertholletia excelsa*	1.34	2.34
Candlenut	*Aleurites moluccana*	1.73	3.63
Cashew nut	*Anacardium occidentale*	5.95	6.62
Chestnut, Chinese	*Castanea spp.*	0.91	0.96
Coconut flesh, old	*Cocos nucifera*	1.21	1.44
Coconut flesh, young	*Cocos nucifera*	0.49	0.51
Coconut milk	*Cocos nucifera*	1.00	1.18
Coconut water	*Cocos nucifera*	0.03	0.05
Lotus seed	*Nelumbo nucifera*	2.88	2.03
Peanut butter	*Arachis hypogea*	1.94	1.86
Sesame seed/Gingelly seed	*Sesamum indicum*	5.05	4.62
Walnut, dried	*Juglans regia*	2.63	2.55
Watermelon seed, black, dried	*Citrullus vulgaris*	5.98	7.86

Each value is the mean of duplicate analysis

TABLE 4
Iron in vegetables as determined by the atomic absorption spectrophotometric and colorimetric methods

English/local name	Scientific name	mg Fe/100 g edible portion	
		AAS method	Colorimetric method
Asam gelugor, shoots	*Garcinia atroviridis*	0.88	0.99
Asparagus, canned	*Asparagus officinalis*	7.06	6.91
Asparagus, fresh	*Asparagus officinalis*	0.53	0.59
Broccoli	*Brassica oleracea*	0.47	0.71
Cemperai	*Champereia griffithii*	1.96	1.90
Chilli, small	*Capsicum annuum*	0.68	1.24
Chives, Chinese	*Allium odorum*	0.62	0.70
Coriander leaves	*Coriandrum sativum*	3.86	2.99
Cucumber, hairy	*Cucumis spp.*	0.15	0.15
Drumstick, fresh pods	*Moringa oleifera*	0.27	0.27
Garlic, bulbs	*Allium sativum*	0.48	0.84
Garlic, plants	*Allium sativum*	0.31	0.42
Gourd, bottle/Calabash	*Lagenaria vulgaris*	0.22	0.27
Kadok, leaves	*Piper sarmentosum*	2.26	1.73
Leek	*Allium porrum*	0.33	0.28
Mushrooms, grey oyster, fresh	–	0.84	0.98
Mustard leaves, Chinese (*Sawi*)	*Brassica juncea*	1.35	1.32
Mustard leaves, Indian (*Kai-coy*)	*Brassica juncea*	1.46	1.45
Parsley	*Petroselinum crispum*	9.90	10.25
Peas, garden, fresh	*Pisum sativum*	0.75	0.78
Salted vegetable	–	1.97	2.05
Seaweed, agar (*Agar-agar*)	–	5.33	5.28
Seaweed, dried	–	22.94	21.71
Spinach, Ceylon	*Basella rubra*	0.88	1.07
Spinach, red	*Amaranthus gangeticus*	2.64	2.44
Spinach (*Bayam duri*)	*Amaranthus spinosus*	1.69	1.05
Waterchestnut	*Scirpus tuberosus*	0.32	0.39
Winter melon / Wax gourd	*Benincasa hispida*	0.14	0.21
Wolfberry leaves	*Lycium chinense*	3.11	2.70
Yam bean (*Sengkuang*)	*Pachyrrhizus erosus*	0.26	0.34

Each value is the mean of duplicate analysis

TABLE 5
Iron in fruits as determined by the atomic absorption spectrophotometric and colorimetric methods

English/local name	Scientific name	mg Fe/100 g edible portion	
		AAS method	Colorimetric method
Avocado	*Persea americana*	0.49	0.57
Banana (*Pisang kelat*)	*Musa sapientium*	0.38	0.53
Binjai	*Mangifera caesia*	0.30	0.30
Cashew apple	*Anacardium occidentale*	0.23	0.27
Custard apple	*Annona squamosa*	0.36	0.36
Date, dried	*Phoenix dactylifera*	0.80	0.76
Duku	*Lansium domesticum*	0.23	0.27
Fruit cocktail in syrup, canned	–	0.58	0.24
Grapefruit	*Citrus paradisi*	0.26	0.22
Jering	*Pithecellobium lobatum*	0.74	0.70
Lime musk (*Limau kasturi*)	*Citrus microcarpa*	0.15	0.17
Lychee	*Litchi chinensis*	0.20	0.21
Mango (*Bacang gelok*)	*Mangifera foetida*	0.22	0.22
Mango (*Kwini*)	*Mangifera odorata*	0.31	0.27
Nutmeg, fresh	*Myristica fragrans*	0.22	0.37
Olive	*Olea europaea*	1.12	1.12
Orange, Mandarin	*Citrus reticulata*	0.20	0.20
Pear, green	*Pyrus communis*	0.20	0.23
Persimmon, dried	*Diospyros kaki*	0.99	1.10
Pineapple syrup, canned	*Ananas comosa*	0.28	0.29
Prunes, dried	*Prunus spp.*	1.07	1.03
Pulasan	*Nephelium mutabile*	0.12	0.17
Rambai	*Baccaurea motleynana*	0.21	0.19
Soursop	*Annona muricata*	0.32	0.37
Strawberry	*Fragaria grandiflora*	0.21	0.20
Water apple	*Eugenia aquea*	0.17	0.17

Each value is the mean of duplicate analysis

TABLE 6
Iron in meat and eggs as determined by the atomic absorption spectrophotometric and colorimetric methods

	mg Fe/100 g edible portion	
	AAS method	Colorimetric method
Beef extract	10.66	12.89
Beef liver, rendang, canned	4.20	4.46
Chicken curry, canned	2.82	3.11
Chicken feet, deboned	0.82	0.85
Chicken gizzard	1.43	1.79
Chicken heart	2.05	2.69
Chicken intestines	0.58	1.11
Corned beef	1.67	1.72
Duck	0.69	1.36
Duck, roasted	0.84	1.28
Duck egg, salted, yolk	8.43	8.27
Duck egg, yolk	3.48	3.50
Mutton curry, canned	3.67	3.82
Ox maw	0.73	0.87
Turtle egg, white	0.36	0.33
Turtle egg, yolk	2.17	2.65

Each value is the mean of duplicate analysis

TABLE 7
Iron in fish and fish products as determined by the atomic absorption spectrophotometric and colorimetric methods

		mg Fe/100 g edible portion	
English/local name	Scientific name	AAS method	Colorimetric method
Anchovy, cleaned	*Stolephorus commersonii*	3.29	3.03
Cuttlefish, dried	*Sepia officinalis*	2.29	2.90
Fish balls	–	0.55	0.50
Fish bladder, dried	–	2.05	1.89
Fish bladder, fried	–	2.63	2.14
Fish curry, canned	–	3.37	3.59
Fish roe	–	1.38	1.80
Fish sauce (*Budu*)	–	3.23	3.17
Hairtail scad, dried	*Megalaspis cordyla*	3.33	3.27
Live crab/Swimming crab	–	0.83	0.95
Oyster sauce	*Ostrea* spp.	0.75	0.50
Oyster	*Ostrea* spp.	4.99	4.96
Prawn paste (*Hay-ko*)	–	21.56	22.31
Sea crab/Blue crab	–	0.50	0.61
Shark's fin, dried	–	3.04	2.52
Shrimp, fermented (*Cincalok*)	–	1.22	1.07
Threadfin, dried	*Polynemus indicus*	1.07	0.96
Yellow banded trevally, dried	*Selaroides leptolepis*	2.05	1.68

Each value is the mean of duplicate analysis

TABLE 8
Iron in miscellaneous foods as determined by the atomic absorption spectrophotometric and colorimetric methods

English/local name	Scientific name	mg Fe/100 g edible portion	
		AAS method	Colorimetric method
Anise seed, dried	*Pimpinella anisum*	100.53	86.19
Cardamon	*Elettaria cardamomum*	20.56	19.85
Cinnamon	*Cinnamomum zeylanicum*	2.17	2.33
Coffee mixture, powder	–	4.47	4.75
Corianda seeds	*Coriandrum sativum*	67.39	51.15
Cumin seeds, black	*Nigella sativa*	42.37	29.91
Cumin seeds, white	*Cuminum cyminum*	42.94	27.46
Curry powder	–	35.82	35.03
Fenugreek seeds	*Trigonella foenum-graecum*	16.74	17.56
Galangal	*Languas galanga*	0.23	0.47
Honey	–	0.62	0.54
Jam, egg (*Seri kaya*)	–	0.35	0.48
Jam, pineapple	–	1.16	1.13
Jelly crystals	–	0.16	0.22
Malted milk powder	–	17.25	17.77
Marmalade	–	0.09	0.12
Milk-based diet supplement, powder	–	3.46	3.47
Pepper, powder, white	*Piper nigrum*	4.59	5.26
Sugar, brown	–	2.76	2.68
Sugar, coconut palm (*Gula Melaka*)	–	0.86	0.73
Sugar cane juice	*Saccharum officinarum*	0.06	0.15
Tamarind paste (*Asam Jawa*)	*Tamarindus indica*	2.95	3.44
Treacle, black	–	14.07	16.94
Yeast, dried	*Saccharomyces cerevisiae*	32.81	27.40

Each value is the mean of duplicate analysis

TABLE 9
Paired t-test of iron concentrations determined by the atomic absorption spectrophotometric and colorimetric methods

Food group	n	Calculated t-statistic	Statistical significance[1]
Cereals and products	14	2.352	S.[2]
Legumes and products	13	0.973	N.S[3]
Nuts and seeds	15	2.033	N.S.
Vegetables	30	0.827	N.S.
Fruits	26	0.376	N.S.
Meat and eggs	16	2.765	S.
Fish and fish products	18	0.191	N.S.
Miscellaneous	24	2.112	S.

[1] at $p < 0.05$
[2] statistically significant
[3] not statistically significant

TABLE 10
Recovery values obtained by the atomic absorption spectrophotometric and colorimetric methods

	AAS method	Colorimetric method
Number of determinations	10	10
Mean ± SD	84.2 ± 9.4%	90.7 ± 11.2%
Coefficient of variation	11.1	12.3

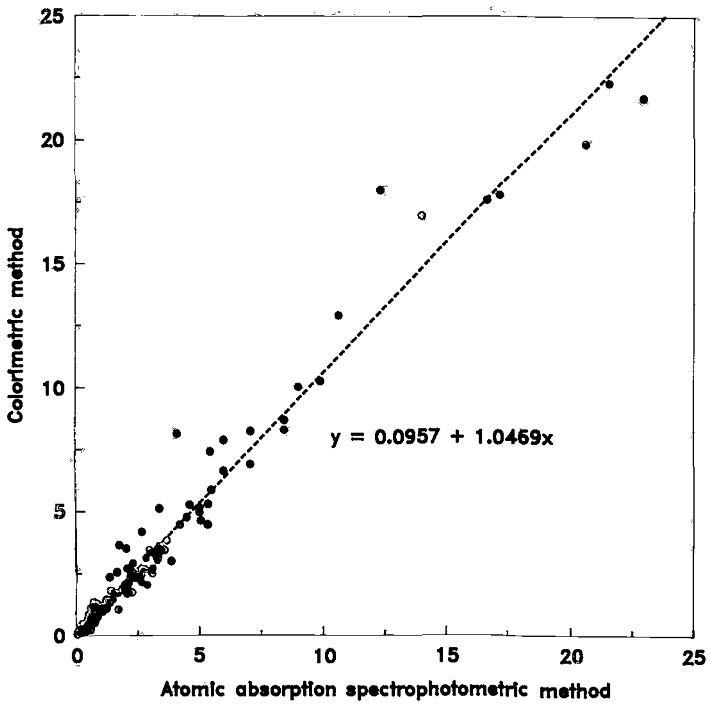

Fig. 1 Iron concentration determined by the AAS and colorimetric methods (mg iron per 100 g edible portion).
n = 150 (results for 6 foods not included)

shows that mean % recovery obtained by the colorimetric method was closer to 100 than that for the AAS method. Statistically however, there was no significant difference ($p < 0.05$) in mean recovery values given by the two methods.

Pooled standard deviation calculated for all the 156 foods studied was 0.39 for the AAS method and 0.63 for the colorimetric method. Comparing the variance obtained for all foods, the observed F-ratio was calculated to be 2.66. Variance for the colorimetric method was thus significantly higher than that for the AAS method ($p < 0.05$).

CONCLUSIONS

Results of this comparative study do not show significantly different iron concentrations for a wide variety of foods between the AAS and the o-phenanthroline colorimetric methods. Both methods were found to give satisfactory recovery values. Variance for the colorimetric method was however slightly higher than that for the AAS method. Either method can, therefore, be used satisfactorily for this analysis. There are, however, other considerations in the choice of a method for use.

In the colorimetric method, several steps are required in preparing solutions for reading in a spectrophotometer. All glassware, chemicals, and water used should be iron-free to prevent contamination to the test solutions. Fortunately, the red colour complex formed is stable for a number of hours. The procedure is also relatively much cheaper, requiring only a low-cost spectrophotometer operating in the visible range. In the hands of a careful worker, the method can perform satisfactorily.

The AAS method, on the other hand, requires the purchase of a high-cost spectrophotometer which is also rather expensive to operate and maintain. It is however, a relatively simpler procedure. The ash solution can be used directly for spraying in the spectrophotometer, after the instrument has been appropriately set up. It would be the method of choice, provided the required budget is available.

Compared to a similar comparative study on calcium determination in foods (Tee *et al.*, 1989), it has been observed that there were more variations between iron content given by the AAS and colorimetric methods. Correlation coefficient for this study was sligthly lower and recovery values were also lower with larger coefficient of variation. Process variation for the colorimetric method was also found to be higher. These relate to the observation that iron determination is rather prone to contamination from the environment. Various precautions, requiring greater degree of care and skill in the laboratory worker, have to be taken to minimise this.

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