Annex 5

ISOTOPE AIDED STUDIES ON THE BIOAVAILABILITY OF IRON AND ZINC FROM HUMAN DIETS CONSUMED IN INDIA *

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Abstract

Total iron, and zinc, in-vitro ionizable iron and soluble zinc were estimated by the chemical and extrinsic isotope tag methods for comparison in various foodstuffs as such, and after processing, and also in diets. It has been observed that the values got were more or less similar by both the procedures. The in-vitro ionizable iron in groundnut was low with low total iron as well. Total iron and ionizable iron were also estimated in commonly consumed breakfast preparations. The effect of tea on ionizable iron when taken along with breakfast was also investigated. It was found that different breakfast preparations varied narrowly with regards to total iron (4.6-7.2 mg) and percent ionizable iron (25%-33%). However, tea had a pronounced effect on ionizable iron resulting in inhibition to various extents. Total and soluble zinc were analyzed in green leafy vegetables and groundnut. Though the total zinc was low and similar in both foodstuffs, the percent soluble Zn was found to be high in green leafy vegetables as compared to groundnut. Tannin and ascorbic acid contents were estimated in a few foodstuffs. Tannin content in green leafy vegetables was found to be about 150 mg. Ascorbic acid concentration was high in cereals (except in rice) and whole pulses. The split pulses (dals) were found to be poor sources of ascorbic acid. Ionizable iron and soluble zinc were found to increase to various extents on processing. Germination was found to increase ascorbic acid, whereas it had no effect on tannin.

1. BACKGROUND AND SCOPE

Iron deficiency anaemia is widely prevalent in our population in spite of seemingly adequate intakes of dietary iron. The main reason for this could be poor absorption. Factors which influence iron absorption are primarily derived from foodstuffs. The well identified inhibitors are phytates and tannins and promoters are ascorbic acid and some reducing agents.

Since iron and zinc tend to move together through the food chain, it is likely that factors which influence bioavailability of iron would also influence zinc. Apart from this, preliminary studies at our Institute indicated that the zinc intakes are around 50% of that recommended for this nutrient. It is therefore possible that zinc deficiency may be common in our population, though no frank symptoms of its deficiency are observed. Only limited studies are available based on the diets composed in the laboratories on the bioavailability of Fe and Zn. No meaningful conclusions can be drawn from such limited

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studies. Hence, detailed research is planned to study the bioavailability of Fe and Zn in the foodstuffs and diets of various regions of the country.

It is also planned to investigate the effect of various inhibitors like tannins and polyphenols and enhancers like ascorbic acid and other organic acids on the bioavailability of iron and zinc. Studies have also been initiated on the bioavailability of iron and zinc in foodstuffs after various kinds of processing and in infant food formulae.

2. EXPERIMENTAL METHODS

Collection of food materials and the estimation of total iron, total zinc, ionizable iron, soluble zinc, tannic acid and ascorbic acid are already described in detail in the previous report (June '92 - Nov.'92).

2.1. Collection of breakfast samples

Six commonly consumed breakfast preparations like idli, uttappam, vada, dosa, upma and poori were collected along with their side dishes from six different hotels in Hyderabad. These items were selected basically in view of the three different methods of preparations like ordinary cooking, open pan frying and deep frying. Thus idli and upma can be categorized as ordinary cooking, dosa and uttappam as open pan frying and vada and poori as deep frying. Idli, uttappam and dosa are preparations of rice in combination with black gramdhal. The proportion of dhal to rice is the same in uttappam and dosa, but idli has higher proportion of dhal to that of rice. Vada is a pulse preparation whereas poori and upma are the preparations of wheat. Duplicate breakfast samples along with side dishes (curry & chutney) with or without tea added were collected. They were weighed, homogenized and lyophilized. Lyophilized samples were used for various estimations.

2.2. Various food kinds of processing

2.2.1. Cooking

The food material was cleaned and washed with deionized water. The sample was cooked under pressure (15 lb/sq inch pressure and at 110°C to 120°C) for five minutes with double the amount of deionized water. Cooked samples were homogenized, lyophilized and powdered.

2.2.2. Roasting

A small amount of foodstuff was cleaned and washed with deionized water and dried in an oven at 50-60°C overnight. The samples were roasted in an open pan till the outer skin split. The sample was then powdered.

2.2.3. Germination

A known amount of whole pulse was cleaned and washed with deionized water and soaked overnight with triple the amount of water. Next day, water was drained and samples were allowed to germinate for 48 hrs between moist layers of filter papers. Samples were homogenized, lyophilized and powdered.

2.2.4. *Malting*

Samples of whole grain were germinated as above. Samples were then dried in an oven at 50-60°C for two days and powdered.

2.3. Measurement of *in-vitro* ionizable iron and zinc using extrinsic tag

The method, in principle, is the same as that described for the chemical procedure, except that a known amount (dpm) of radioactive $^{59}FeCl_3$ or $^{65}ZnCl_2$ was added after pepsin HCl treatment (pH 1.35) of the food material. The radioactivity was measured in an aliquot of supernatant after protein precipitation. These counts were expressed after appropriate corrections as percentage of the total counts added initially, which indicates the percent ionizable iron. Radioactivity was counted in a gamma counter (Packard auto gamma 500 model).

3. RESULTS

The total and % *in-vitro* ionizable iron in groundnut were 3.81 ± 0.06 mg/100g and 3.67% respectively. The data on total and ionizable iron and the effect of tea on ionizable iron are given in table I and figure 1.

3.1. Total and ionizable Fe and effect of tea on ionizable iron in breakfast preparations

The total iron in various breakfast preparations varied from 4.40 to 6.96 mg/100g dry weight and with an ionizable form being 25% to 33%. Percent ionizable and total Fe did not vary much with the type of preparation of the breakfast but had a bearing on the ingredients of the preparation. There was no difference in the percent ionizable Fe in upma and poori (26% in both) but there was a difference in the total Fe (6.5 mg in upma and 4.8 mg in poori) though both the preparations are made from wheat.

The inhibitory effect of tea on *in-vitro* ionizable iron varied from 25% to 53% with various breakfast preparations (Fig. 1), the lowest being observed with poori, a wheat preparation and the highest inhibition with uttappam, a rice and dhal preparation. The inhibitory effect of ionizable iron observed with wheat breakfast preparations (poori and upma) was significantly ($P < 0.001$) lower as compared with other breakfast preparations (rice and dhal preparations).

3.2. Effect of food processing on *in-vitro* ionizable Fe

Various kinds of processing of food materials like cooking, germination, roasting and malting on ionizable iron were investigated and the data are presented in table I.

It can be seen from the table that processing has resulted in the increase in ionizable iron to various extents in all the foods tested. However, the increase was higher on roasting and malting than cooking and germination.

3.3. Ascorbic acid and tannic acid content on processing

Tannin content in various foodstuffs on processing is given in table III and ascorbic acid content on germination is given in table IV. It can be seen from the table that tannin content did not vary much in many of the foods with various kinds of processing. However, ascorbic acid content on germination increased two to four fold in the pulses tested.

3.4. Tannin content in green leafy vegetables

Table V gives the values of tannin in various green leafy vegetables commonly consumed. The tannin content varied from 183.5 to 226.1 mg/100g except gongura which had low tannin content of only 80.1 mg/100g.

3.5. Ascorbic acid content in various foodstuffs

The results of ascorbic acid content in cereals, whole pulses, split pulses are given in Table VI. Ascorbic acid content was lowest in split pulses as compared to cereals, (except rice) and whole pulses. Whole pulses and cereals had more or less similar values of ascorbic acid.

3.6. Total and soluble Zn in green leafy vegetables, groundnuts and in various processed foodstuffs

The total and soluble Zn contents of groundnut and green leafy vegetables are given in table VII. Total Zn as well as soluble Zn in green leafy vegetables varied narrowly from one another. Though the total Zn in groundnuts is in the same range as found in green leafy vegetables, the soluble Zn was significantly lower in groundnuts as compared to green leafy vegetables.

Total and soluble zinc following various kinds of processing are given in table VIII. It can be seen that cooking and malting did not have much effect on soluble Zn, whereas on germination, the soluble Zn has increased considerably.

3.7. Comparison of chemical and isotopic method of *in-vitro* ionizable iron and soluble Zn in various foods

A large number and variety of foodstuffs and a number of breakfasts were analyzed for *in-vitro* ionizable iron both by the chemical as well as by the extrinsic isotope methods and the results are compared and given in figures 2 and 3 respectively. The values of percent ionizable iron by both methods were found to be similar and the correlation coefficient was found to be 0.986 in foodstuffs and 0.925 in the breakfast samples. Similar methods were followed for soluble Zn in various foodstuffs, and the results were found to be comparable. The data is given in figure 4 and the correlation coefficient was found to be 0.984.

4. CONCLUSIONS

The results clearly demonstrated that various kinds of processing of foodstuffs resulted in marginal to high increases in ionizable iron; the highest was observed with malting. Food processing did not alter much the tannin content but remarkably increased ascorbic acid on germination. Higher ionizable iron observed on malting, a process involving germination, may be attributable to increased ascorbic acid content. High *in-vitro* ionizable iron in green leafy vegetables may be attributable to high ascorbic acid content of about 100 mg in spite of having 150 mg of tannin, which may be considered as high. Total ascorbic acid content was marginal in cereals and whole pulses, but was low in rice and split pulses.

Total zinc and soluble zinc in green leafy vegetables indicated that, though the total Zn was low, soluble Zn was high and was around 65%. Soluble Zn on processing indicated increased availability with processing particularly with germination.

The total iron intake from various breakfasts varied from 4.6 to 7.4 mg, about 20% of the RDA for Fe. Tea had pronounced inhibitory effect on the ionizable Fe of the various breakfasts, maybe because of the high tannin content in tea. Similar observations were also made earlier. The inhibitory effect of tea was less on wheat based breakfasts as compared to other breakfasts.

5. FUTURE PLAN OF WORK

(1) Bioavailability studies of iron using the extrinsic tag method and a whole body counter in human volunteers will be initiated shortly.

(2) The regional diets planned to be investigated include the following staples:
- (i) rice
- (ii) wheat
- (iii) jowar
- (iv) ragi
- (v) maize
- (vi) bajra

Each of the above staples will be studied with the side dish combination of (i) pulses, (ii) meat, (iii) GLV, (iv) vegetables (v) fish and (vi) milk products.

(3) Infant food formulae will be studied using *in-vitro* methods for the availability of Fe and Zn.

Three papers are now in preparation for publication.

TABLE I.

BREAKFAST ITEMS	MAJOR INGREDIENTS	METHOD OF PREPARATION	TOTAL IRON		PERCENT IONIZABLE IRON
			mg / TOTAL BREAKFAST	mg / 100 g DRY WT.	
1. Idly + chutney + Sambar	Parboiled Rice + Blackgram Dhal	Steaming	4.63 ± 0.50	5.65 ± 0.41	25 ± 2.5
1a. Idly + Chutney + Sambar + Tea			5.20 ± 0.57	5.25 ± 0.14	12 ± 2.0
2. Uttappam + Khurma + Chutney	Rice + Blackgram Dhal + Onions	Open pan frying	7.18 ± 0.51	4.82 ± 0.34	33 ± 2.0
2a. Uttapam + Khurma + Chutney + Tea			7.44 ± 0.53	4.59 ± 0.34	16 ± 1.8
3. Dosa + Curry + Chutney + Sambar	Rice + Blackgram Dhal	Open pan frying	5.72 ± 0.48	4.40 ± 0.37	30 ± 5.1
3a. Dosa + Curry + Chutney + Sambar + Tea			6.56 ± 0.53	4.49 ± 0.36	17 ± 2.1
4. Vada + Chutney + Sambar	Blackgram Dhal	Deep fat frying	6.89 ± 0.75	6.96 ± 0.75	25 ± 2.5
4a. Vada + Chutney + Sambar + Tea			6.84 ± 0.76	6.16 ± 0.68	15 ± 1.5
5. Upma + Chutney	Broken fine wheat	Ordinary cooking	4.68 ± 0.53	6.50 ± 0.72	26 ± 2.6
5a. Upma + Chutney + Tea			5.32 ± 0.56	6.26 ± 0.66	17 ± 2.2
6. Poori + Khurma	Wheat flour	Deep fat frying	5.24 ± 0.58	4.76 ± 0.53	26 ± 1.8
6a. Poori + Khurma + Tea			5.96 ± 0.81	4.62 ± 0.62	20 ± 2.1

Values are Mean ± SE of 6 samples

TABLE II. EFFECT OF VARIOUS PROCESSING ON IONIZABLE IRON

FOODS	TOTAL Fe mg/100g	PERCENT IONIZABLE Fe
	Cooking	
Rice	0.60 ± 0.03 (0.61 ± 0.09)	33.33 ± 1.60 (29.5 ± 4.75)
Lentil dal	6.01 ± 0.20 (6.31 ± 0.21)	50.00 ± 1.16 (31.70 ± 1.74)
Redgram dal	3.63 ± 0.25 (3.62 ± 0.12)	36.64 ± 1.40 (23.50 ± 0.83)
	Germination	
Greengram	6.10 ± 0.13 (6.12 ± 0.12)	11.70 ± 0.50 (6.53 ± 1.31)
Bengalgram (Desi)	5.63 ± 0.26 (5.71 ± 0.20)	7.46 ± 0.53 (6.30 ± 0.35)
Bengalgram (Kabuli)	5.62 ± 0.44 (5.62 ± 0.40)	26.00 ± 2.1 (21.71 ± 0.53)
	Roasting	
Groundnut	3.82 ± 0.12 (3.81 ± 0.06)	6.81 ± 0.18 (3.67 ± 0.26)
Bengalgram (Desi)	5.60 ± 0.31 (5.71 ± 0.20)	15.00 ± 2.10 (6.3 ± 0.35)
Bengalgram (Kabuli)	5.55 ± 0.45 (5.62 ± 0.40)	42.70 ± 6.00 (21.71 ± 0.53)
	Malting	
Ragi	5.84 ± 0.25 (7.85 ± 1.66)	14.90 ± 0.70 (4.84 ± 0.25)
Bajra	7.06 ± 1.20 (8.00 ± 0.92)	34.30 ± 2.41 (13.75 ± 2.12)
Wheat	5.83 ± 0.35 (5.87 ± 0.26)	11.66 ± 1.40 (7.70 ± 1.36)
Sorghum	3.30 ± 0.20 (4.40 ± 0.50)	26.36 ± 2.45 (15.10 ± 0.90)
Maize	2.87 ± 0.13 (2.73 ± 0.14)	34.84 ± 1.74 (25.30 ± 1.46)

Values are mean ± SEM of 4 samples

Figures in parenthesis indicate the values of unprocessed samples.

TABLE III. TANNIN CONTENT IN VARIOUS PROCESSED FOODSTUFFS

FOODSTUFFS	mg/100g
Malting	
Ragi	515.1 ± 102.00 (598.2)
Wheat	19.7 ± 0.96 (21.9)
Bajra	12.4 ± 1.40 (12.9)
Jowar	0.0 ± 0.00 (3.3)
Maize	19.5 ± 0.97 (24.1)
Roasting	
Bengalgram (Desi)	42.8 ± 6.33 (41.4)
Bengalgram (Kabuli)	25.8 ± 2.57 (33.1)
Groundnut	355.0 ± 0.13 (765.1)
Germination	
Greengram	104.5 ± 9.40 (100.4)
Bengalgram (Desi)	41.4 ± 8.01 (41.4)
Bengalgram (Kabuli)	22.9 ± 3.18 (33.1)
Cooking	
Rice	ND (-)
Lentil Dal	9.55 ± 0.55 (41.8 ± 3.51)
Redgram Dal	8.55 ± 2.31 (44.00 ± 2.36)

Values are mean ± SEM of 4 samples

Figures in parenthesis indicate the values of unprocessed samples

TABLE IV. ASCORBIC ACID IN GERMINATED PULSES (mg/100g)

FOODSTUFFS	mg/100g
Bengalgram (Desi)	17.8 ± 0.55 (5.6)
Bengalgram (Kabuli)	18.0 ± 0.52 (5.5)
Greengram	47.5 ± 0.70 (29.8)

Values are mean ± SEM of 4 samples

Figures in parenthesis indicate the values of unprocessed samples

TABLE V. TANNIN CONTENT IN GREEN LEAFY VEGETABLES

FOODSTUFFS	mg/100g
Agathi	183.5 ± 1.35
Bachali	192.3 ± 6.20
Fenugreek	226.1 ± 8.32
Gongura	80.1 ± 7.18
Amaranth	198.5 ± 12.90
Spinach	185.3 ± 12.26

Values are mean ± SEM of 4 samples

TABLE VI. ASCORBIC ACID CONTENT IN VARIOUS FOODSTUFFS

FOODSTUFFS	mg/100g
Cereals	
Jowar	14.1 ± 0.23
Wheat	16.0 ± 0.61
Maize	20.3 ± 0.66
Ragi	32.4 ± 1.06
Bajra	31.7 ± 1.10
Rice	1.5 ± 0.13
Whole pulses	
Blackgram (kabuli)	49.9 ± 0.98
Bengalgram (Desi)	15.5 ± 0.31
Greengram	15.6 ± 0.23
Rajma	51.3 ± 1.34
Lentil	27.0 ± 0.85
Peas	18.6 ± 0.74
Split pulses	
Blackgram	3.6 ± 0.24
Bengalgram	5.3 ± 0.20
Greengram	8.3 ± 0.24
Lentil	2.8 ± 0.14
Redgram	6.73 ± 0.23

Values are mean ± SEM of 6 samples

TABLE VII. TOTAL AND SOL JBLE Zn IN GREEN LEAFY VEGETABLES AND GROUNDNUT

FOOD ITEMS	TOTAL Zn mg/100g	SOLUBLE Zn %
Bachali	3.5 ± 0.11	66.9 ± 1.17
Agathi	2.9 ± 0.06	64.4 ± 1.83
Gongura	2.6 ± 0.57	67.2 ± 2.05
Fenugreek	3.8 ± 0.15	67.0 ± 1.37
Amaranth	3.8 ± 0.15	53.3 ± 0.96
Spinach	2.8 ± 0.15	63.3 ± 0.56
Groundnut	4.2 ± 0.28	7.2 ± 0.64

Values are mean ± SEM of 4 samples

TABLE VIII. TOTAL AND SOLUBLE Zn FOLLOWING VARIOUS KINDS OF FOOD PROCESSING

FOOD ITEMS	TOTAL Zn mg/100g	SOLUBLE Zn %
Cooking		
Rice	1.0 ± 0.03 (1.2)	13.2 ± 0.60 (11.0)
Lentil dal	2.1 ± 0.11 (2.2)	54.1 ± 0.82 (36.2)
Redgram dal	2.1 ± 0.08 (2.4)	46.1 ± 1.27 (45.4)
Germination		
Greengram dal	2.3 ± 0.06 (2.5)	32.5 ± 1.23 (21.3)
Bengalgram dal (Kabuli)	2.1 ± 0.06 (2.4)	51.1 ± 3.03 (35.1)
Bengalgram dal (Desi)	3.1 ± 0.09 (3.5)	51.1 ± 1.34 (32.9)
Malting		
Wheat	3.2 ± 0.15 (3.2)	2.7 ± 0.31 (1.6)
Maize	2.2 ± 0.13 (2.7)	10.8 ± 0.17 (10.8)
Sorghum	1.3 ± 0.13 (1.5)	3.8 ± 0.93 (4.6)
Ragi	1.4 ± 0.05 (1.6)	11.5 ± 1.52 (13.5)
Bajra	3.5 ± 0.12 (4.4)	15.6 ± 0.32 (13.8)

Values are Mean ± SEM of 4 samples

Figures in parenthesis indicate values of unprocessed foods

FIG. 1. Effect of tea on ionizable iron in breakfast preparations

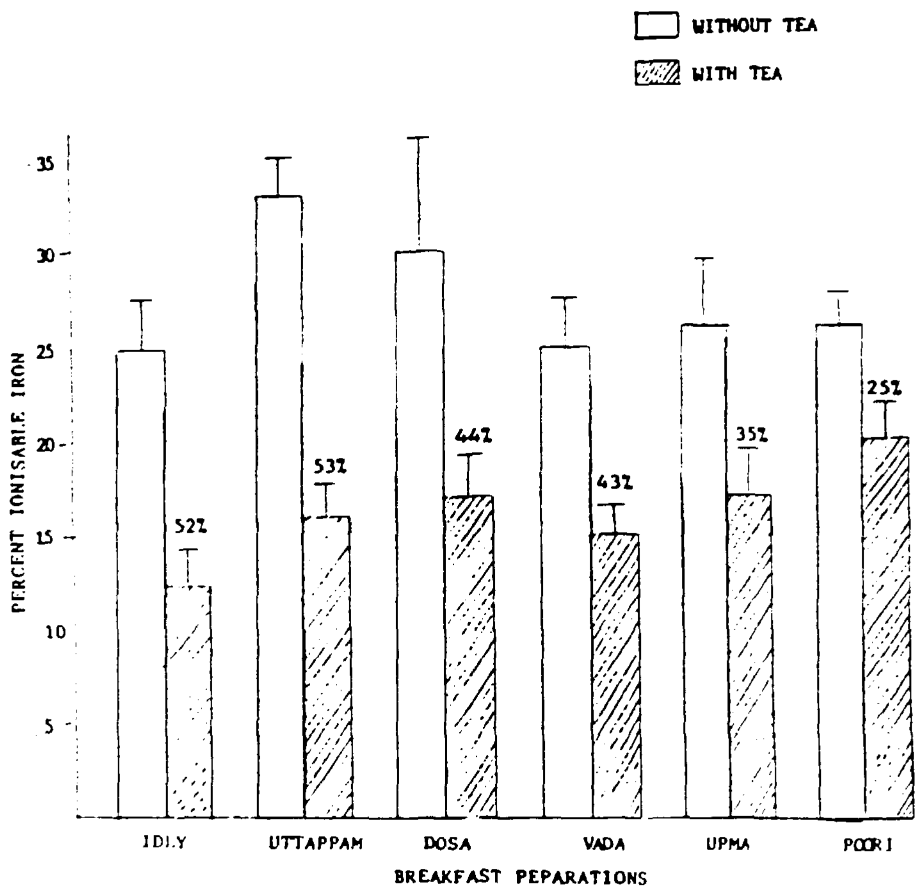

FIG. 2. Comparison of chemical and isotopic methods for assessing ionizable iron in foodstuffs

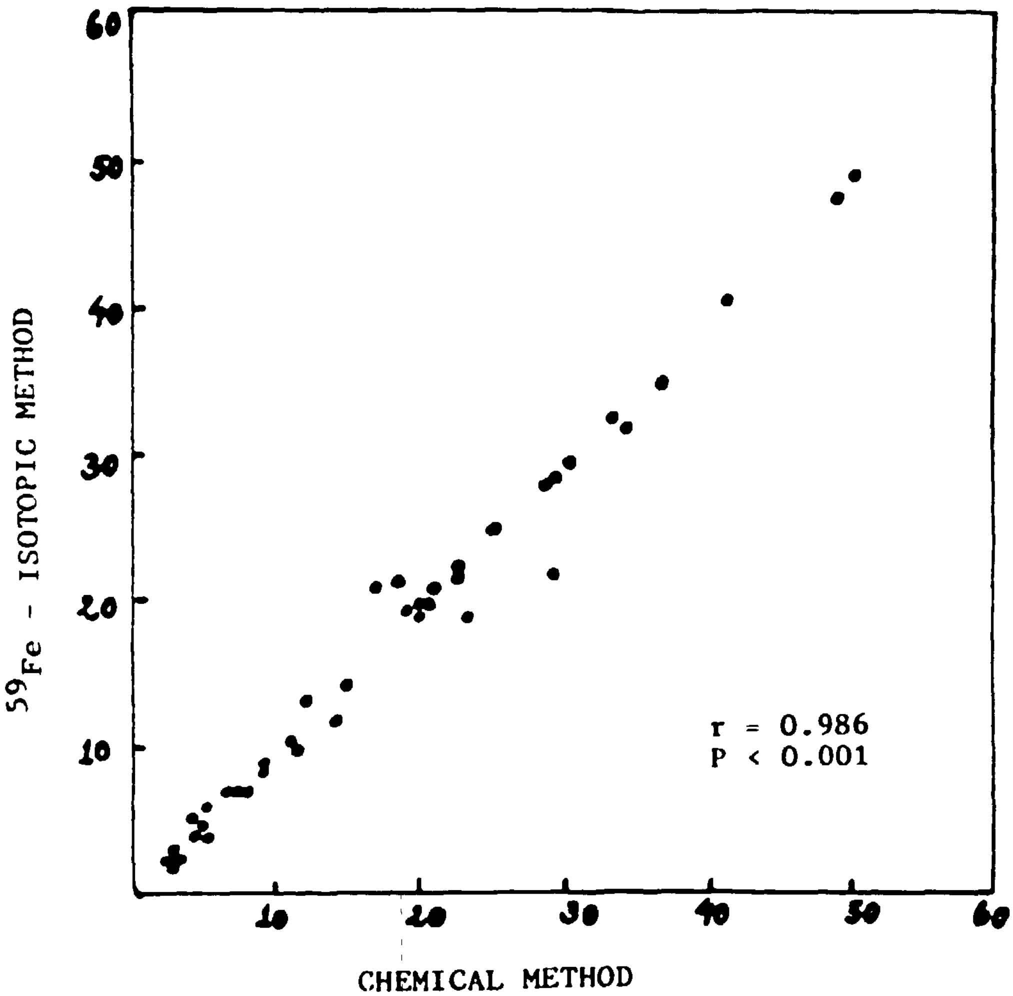

FIG. 3. Correlation between the chemical and isotopic methods for assessing ionizable iron in breakfast preparations

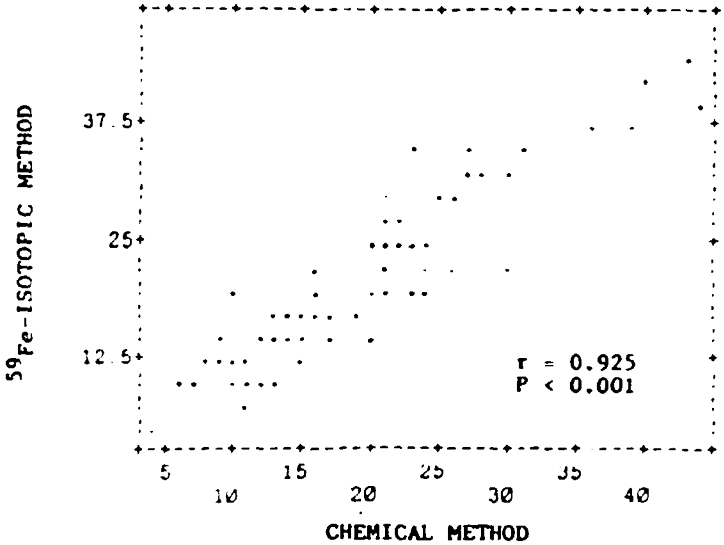

FIG. 4. Correlation between the chemical and isotopic methods for assessing soluble zinc in foodstuffs