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Trial of ferrous glycine sulphate in the fortification of common salt with iron

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Ferrous glycine sulphate, an organic complex of glycine and ferrous sulphate, was tested as a source of iron to produce iron fortified salt. The dry mixing was found superior to the spray mixing technique. The fortified salt retained the colour of common salt. The iron distribution was uniform, approximately 1000 ± 50 ppm. The iron stability of the fortified salt during long storage (1 year) was satisfactory and the iron was available in the ferrous form. The available iron from the fortified salt, measured by the *in vitro* method, was 70% which was 3 times higher than ferrous sulphate fortified salt. The acceptability of the fortified salt was satisfactory. Various food items prepared using the fortified salt were indistinguishable from those containing unfortified salt in colour, taste, flavour or texture. Factory production was smooth. The chemical cost, of iron fortification was the same as that of the earlier formulations of iron fortified salt. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Iron deficiency anaemia (IDA) is a major nutritional problem in the developing world and affects all segments of the population. It is particularly severe in infants and child bearing women (WHO, 1990; Baynes & Bothwell, 1990). The prevalence of IDA in the Indian population is high and ranges from 40 to 70% depending upon age and sex. Food fortification is generally considered the best long- term approach for combating IDA (WHO, 1975; INACG, 1977; Cook & Russer, 1983; DeMaeyer, 1989; MacPhail & Bothwell, 1989; Hurrell & Cook, 1991). Common salt fulfils all the requirements of an ideal vehicle for iron fortification in India. The usefulness of iron fortified salt (IFS) in controlling IDA has been demonstrated by population studies in the country (Report, 1982). Several formulae have been reported for the production of IFS using stabilisers and absorption promoters for iron (Narasinga Rao & Vijayasarathy, 1975, 1978; Ranganathan, 1992). However, the formula using ferrous sulphate and sodium hexametaphosphate was shown to exhibit better iron absorption (Ranganathan, 1992) and also was free from any discolouration and other problems in the factory (Ranganathan *et al.*, 1993). Nevertheless, no IFS formula has been reported using only the iron compound. This is mainly because of the fact that the ferrous sulphate is easily oxidised to the less utilisable ferric sulphate (The Merck Index, 1989). Fortification of common salt with an iron compound alone will have several advantages.

Ferrous glycine sulphate (FGS), an organic complex of ferrous sulphate and glycine, has been shown to be an effective oral haemmatinic in controlling IDA in children (Todd, 1958), adults (Pomeranze & Gadek, 1957; Coplin *et al.*, 1991; Barnes, 1960; Krantz & Carr, 1969) and pregnant women (Jennison, 1958; Cameron, 1974; Piccinini, 1961; Rummel & Candon, 1956; Rummel, 1960). The presence of glycine maintains the ferrous iron in a soluble form throughout the entire pH ranage of the gastro-intestinal tract. For this reason it is reliably absorbed (Cameron, 1974). Direct toxicity to the gastric mucosa by ferric iron is minimised by two factors: 1) the very low ferric iron content and 2) the fact that ferric iron present will be maintained in a soluble and non-irritant form by glycine. The low incidence of side-effects and complaints from patients underline the excellent tolerance and acceptability of the FGS (Cameron, 1974; Coplin *et al.*, 1991). The FGS complex passes through the intestinal walls both rapidly and in high concentration (Jacobi *et al.*, 1956; Hegsted *et al.*, 1949). When exposed to the atmosphere for three months (Jennison, 1958) FGS showed no oxidation to ferric iron; nor was it precipitated in alkaline solution even at a pH much higher than the pH found in the human intestinal region, whereas ferrous sulphate produced heavy precipitate (Arden, 1950).

Therefore, attempts were made to fortify common salt only with FGS without adding any stabiliser and/or absorption promoter for iron.

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MATERIALS AND METHODS

Materials

Salt
Edible grade solar crystal salt was obtained from Tuticorin (India).

Chemicals
FGS powder (I.P. Grade) was supplied by M/s. Medcell Laboratories Pvt. Ltd, Madras (India). Ferrous sulphate heptahydrate (I.P. Grade) was supplied by M/s. Abhyudaya Chemical and Scientific Corporation, Hyderabad (India).

Methods

Fortification process
Common salt was crushed to a coarse powder (mesh size # 10). FGS at different levels to give iron levels of 125, 250, 500, 750 and 1000 ppm was added to 1 kg of crushed salt. In the 'spray mixing' process the FGS was dissolved in water and sprayed over the salt and mixed thoroughly. In the 'dry mixing' process, FGS powder was mixed with 0.1 kg of the salt and the 'pre-mix' was added to the remaining 0.9 kg of salt and mixed well to achieve uniform distribution of iron.

Storage and stability
FGS stored in plastic bags was tested every month for its ferrous and ferric content for 1 year.

The IFS samples packed in 1 kg LDPE bags were stored at normal room conditions and also at high humidity (RH 78%) in a humidity chamber. All the samples were tested for ferric iron and total iron by the thiocyanate method (Wong, 1965); the ferrous iron was measured by the AOAC method (AOAC, 1990) every month for one year. At each time point samples were drawn randomly according to the standard ISI procedure (ISI Hand Book, 1978).

Availability of iron
Iron available from the IFS was tested by the *in vitro* method (Narasinga Rao & Prabhavathi, 1978). Ionisable iron was determined in the pH7.5 filtrate by the AOAC method (AOAC, 1990). In the same fashion, common salt fortified with ferrous sulphate alone was also tested for available iron every month by the *in vitro* method.

Table 1. Ferric content of ferrous glycine sulphate

Batch no.	Ferric content (%)		
	Initial	1 year	Difference
FG—110	2.0	4.2	2.2
FG—211	2.2	3.9	1.7
FG—288	2.4	3.4	1.0
FG—366	1.6	3.1	1.5

Acceptability trial
The IFS and the unfortified common salt were distributed to 40 families drawn from different socio-economic backgrounds. The families did not know which salt was fortified. The participants were asked to rate different quality attributes by assigning scores specified ranging from 1 to 5, namely 5 = very good, 4 = good, 3 = fair, 2 = poor and 1 = very poor. Statistical analysis was done using one way analysis of variance to test the difference between mean scores allotted.

Factory trials
Large scale production of IFS was tested in a local salt factory. A stainless steel ribbon blender with a capaciy to hold 100 kg salt was used. FGS was added at 0.5% level to give an iron concentration of 1000 ppm. In the dry mixing process, 500 g FGS powder was mixed with 10 kg crushed salt and mixed well. The 'pre-mix' was added to 90 kg crushed salt taken in the ribbon blender which was rotating. The mixing was continued for 10 min. In the 'spray mixing' process, 500 g FGS powder was dissolved in minimum quantity of water (750 ml) and the resulting solution was sprayed over 100 kg crushed salt taken in the ribbon blender. The mixing was done for 10 min. After the fortification, samples of IFS produced by the above two methods were tested for the uniform distribution of iron.

RESULTS

Stability of FGS

On analysing different batches of FGS, it was found that the oxidation of ferrous iron to ferric iron after 1 year of storage was less than 2%. The initial ferric content observed at the time of manufacture did not influence the oxidation process (Table 1). The iron content of FGS was 17.2%.

Stability of IFS

The effect of storage on the stability of iron in the IFS was satisfactory. The iron (ferrous) remained almost at the initial level until one year. Iron stability was satisfactory at high humidity also (Table 2).

Table 2. Iron content of ferrous glycine sulphate fortified salt

Group	FGS (%)	Iron (ppm)					
		Initial		6 months		1 year	
		RT	HH	RT	HH	RT	HH
1	0.06	125	125	120	119	123	124
2	0.13	250	250	254	249	251	250
3	0.25	500	500	510	515	500	505
4	0.38	750	750	745	740	745	740
5	0.50	1000	1000	1010	1008	1002	1000

FGS = Ferrous glycine sulphate.
RT = Room temperature.
HH = High humidity (78% RH).

Table 3. Available iron of ferrous glycine sulphate fortified salt at different iron levels

Iron (ppm)	Available iron (%)		
	Initial	6 months	1 year
125	91	90	90
250	90	92	89
500	80	79	81
750	75	76	74
1000	70	67	69

Discolouration of IFS

The IFS produced by the 'spray mixing' process turned light brown at 125–250 ppm of iron; brown at 500–750 ppm and dark brown at 1000 ppm. No such discolouration was observed in the IFS produced by the 'dry mixing' process; the IFS retained the same colour of the unfortified salt.

Iron distribution

Analysis of several batches of IFS produced by the 'dry mixing' process showed uniform distribution of iron and the variation was 2–4%.

Availability of iron

The IFS showed satisfactory levels of iron available when tested by the *in vitro* method. It was 90% at 125–250 ppm iron; 80% at 500 ppm; 75% at 750 ppm and 70% at 1000 ppm iron. There were no changes in these values even after 1 year of storage (Table 3).

The available iron from ferrous sulphate fortified salt was 24%, but it decreased rapidly within 2 months. When compared to ferrous sulphate, the FGS showed higher available iron (Table 4).

Acceptability trial

The IFS was acceptable to the families who participated in the study. Their breakfast consisted of idli, dosa, upma, pongal, vada and puri. They had rice or wheat lunch along with vegetarian or non-vegetarian dishes. Mango and lemon pickles were used. Vegetables such as beans, ladies finger, potato, brinjal, tomato, cucumber and fruits (apple, guava and pineapple) were also used.

The results did not show any change in the organoleptaic properties of foods prepared with IFS as ascertained by the scores given by the participants (Table 5). The IFS was well accepted in the daily cooking. There was no complaint of the foods prepared with IFS.

Table 4. Comparison of available iron of ferrous glycine sulphate fortified salt with ferrous sulphate fortified salt

Iron source[a]	Available iron (%)			
	Initial	3 months	6 months	1 year
Ferrous sulphate	24	4	3	2
Ferrous glycine-sulphate	70	70	67	69

[a]Iron = 1000 ppm.

Factory trials

The 'spray mixing' process was problematic and found unsuitable for the production of IFS since the IFS turned brown soon after fortification and the colour persisted even after several months. There was no discolouration of the IFS produced by the 'dry mixing' process and there was no production problem in the factory. The IFS was free-flowing and retained the colour of the unfortified salt. Uniform iron distribution was observed (1000 ± 50 ppm). The pH of a 5% aqueous solution of the IFS was 3.4 when several samples were tested in the factory.

DISCUSSION

Food fortification is a suitable approach to overcome IDA in India. Common salt is considered to be the most ideal vehicle for the iron fortification in the country as it satisfies all the criteria of an ideal vehicle. Therefore, salt fortification with iron is advocated being a simplest, cheapest and most effective method (Pichmani Subramanian, 1989). The earlier formulae of IFS developed in the country using ferric orthophosphate and sodium acid sulphate (Narasinga Rao & Vijayasarathy, 1975) or ferrous sulphate, orthophosphoric acid and sodium acid sulphate (Narasinga Rao & Vijayasarathy, 1978) were not successful in the factories due to the yellow colouration of the IFS and the corrosion of the plant from the acidic nature of the sodium acid sulphate and orthophosphoric acid. These problems were solved in the formula using ferrous sulphate and sodium hexametaphosphate as stabiliser-cum-absorption promoter (Ranganathan, 1992). However, until now no IFS was produced using only the iron compound and without adding any stabiliser or absorption promoter.

FGS is a greyish brown free flowing powder, which is odourless. It has a sweet, mild astringent taste and excellent acceptability. Unlike ferrous sulphate, there is no lingering metallic taste on the tongue.

Table 5. Acceptability of iron fortified salt in foods: mean scores assigned to foods prepared with iron fortified salt and common salt

Food	Colour and appearance		Flavour		Taste		Overall quality	
	IFS	CS	IFS	CS	IFS	CS	IFS	CS
Breakfast	4.9	4.9	4.6	4.6	4.8	4.7	4.9	4.9
Lunch/dinner	4.9	4.8	4.8	4.7	4.9	4.8	4.8	4.7
Snacks	4.8	4.8	4.9	4.9	4.8	4.6	5.0	4.9
Vegetables (cooked)	4.7	4.7	4.8	4.7	4.9	4.9	5.0	4.8

IFS = Iron fortified salt.
CS = Common salt.

A great number of iron compounds are used in the treatment of iron deficiency. It is very difficult to compare the absorbability of iron compounds. It is also difficult to get an objective measurement of the therapeutic value of different iron compounds. A study by Brise & Hallberg (1962) is often quoted in this regard. Although several iron compounds were compared for absorbability of iron in this study (Brise & Hallberg, 1962), the basic flaw in this study was that 10 mg of ascorbic acid was used with every 30 mg of ferrous sulphate in order to prevent the oxidation of the ferrous iron. Hence the results showed that no iron compound was better absorbed than ferrous sulphate. Perhaps, when that paper was published the potential role of ascorbic acid as a strong absorption promoter of iron was not understood.

FGS is stable and resists oxidation. It is extensively used as an oral haematinic. Studies in children, adults and pregnant women showed better iron absorption and remarkable freedom from side effects. In 54 patients with an average haemoglobin of 9 g/100 ml, after 28 days of medication with FGS tablet (2 tablets, 3 times daily between meals, 40 mg ferrous iron per tablet), the average haemoglobin had increased to 14.5 g/100 ml (Pomeranze & Gadek, 1957). When given to 32 children aged 4 weeks–11.5 years, FGS preparation was readily accepted, well tolerated and produced an average daily rise of 1% haemoglobin (Todd, 1958). Several studies have been reported proving the efficacy of FGS in treating IDA by oral route (Jennison, 1958; Barnes, 1960; Karantz & Carr, 1969; Cameron, 1974; Coplin *et al.*, 1991).

The FGS showed satisfactory results in the present study for its excellent use in salt fortification. There was no discolouration of IFS produced by dry mixing; iron was available in an utilisable form even after 1 year. The percent available iron determined by the *in vitro* method was 70%. It was 24% in ferrous sulphate fortified salt; 40% in the IFS produced from ferrous sulphate and sodium hexametaphosphate (Ranganathan, 1992); 38% in the iron and iodine fortified salt made from ferrous sulphate, sodium hexametaphosphate and potassium iodide (Narasinga Rao, 1994). Hence the FGS–IFS has 2–3 times higher available iron when compared to the earlier IFS formulations. Furthermore, no change was observed in the available iron after one year also.

The per cent available iron as determined by the *in vitro* method in a number of diets have been shown to correlate highly with the per cent iron absorption from the same diets in adults. Several studies have shown that the *in vitro* available iron is a good measure of bioavailable iron (Narasinga Rao & Prabhavathi, 1978; Schricker *et al.*, 1981; Forbes *et al.*, 1989). In the ferrous sulphate fortified salt, the *in vitro* available iron was 24% and the corresponding *in vivo* iron absorption was 4.6% (Narasinga Rao & Vijayasarathy, 1975); in the ferrous sulphate, sodium hexametaphosphate fortified salt, the *in vitro* and *in vivo* iron values were 40% and 7% respectively (Ranganathan, 1992); the two values were 38% and 6.3% in the iron and iodine fortified salt (Narasinga Rao, 1994). In all the cases, the *in vivo* iron absorption was approximately 18% of the available iron as determined by the *in vitro* method. Therefore, the FGS–IFS with an *in vitro* available iron of 70% is likely to give higher iron absorption.

The acceptaility trial and the factory trial were satisfactory. The factory trial showed that the manufacture of IFS was cost effective since the fortification involved the addition of only FGS and no other additional chemical. The cost of FGS is roughly three times higher than ferrous sulphate, but the chemical cost of fortification remained the same as that of the earlier formulations of IFS since the addition of stabilisers and absorption promoters are not involved.

Thus, FGS is found to be a suitable source of iron, without the need for stabiliser or absorption promoter, for the production of IFS.

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