Iodine stability in iodized salt dual fortified with microencapsulated ferrous fumarate made by an extrusion-based encapsulation process

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ARTICLE INFO

ABSTRACT

The development of a novel, extrusion-based process for making microencapsulated ferrous fumarate for salt double fortification has been reported earlier. This paper focuses on the results of a one-year storage test, specifically the stability of both iodine and ferrous iron in the double fortified salt (DFS) samples prepared using optimal formulations of the iron premix. The study was devised to test the effectiveness of the encapsulation system in the prevention of interaction between ferrous fumarate and iodine, and preservation of the iodine in iodized salt. The results confirmed that direct iodine–iron interaction occurred in the DFS samples when the iron compound was added without proper coating. However, when an appropriately encapsulated iron premix was used, the interaction could be completely prevented. The extrusion-based process has proven to be an effective approach to producing a stable, bioavailable iron premix, suitable for incorporation into iodized salt for combating iodine and iron deficiencies.

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1. Introduction

Universal salt iodization has been practised in over one hundred countries worldwide in the past five decades, due to the efforts of the Kiwanis Clubs, International Council for the Control of Iodine Deficiency Disorders (ICCIDD), and UNICEF (Salt Institute website, 2008). Salt is an ideal vehicle for micronutrient fortification as it is one of the few commodities that are universally consumed, at a fairly constant rate, by almost all segments of a community irrespective of economic level (Mannar and Dunn, 1995). In addition, it is almost always centrally processed and is open to a simple fortification technique, which makes the program affordable.

Potassium iodate (KIO_3) is commonly used for salt iodization in developing countries, where unrefined salt is consumed and storage conditions are often harsh with high temperature ($\sim 40\,°C$) and high humidity (60–100% relative humidity). As a result, impure iodized salt can lose most of the added iodine during extended storage (Diosady et al., 1997, 1998).

There have been many attempts to use salt for delivery of other micronutrients, such as iron, zinc, calcium, vitamin A, and folic acid (Lotfi et al., 1996). Several attempts at double fortifying salt with iron and iodine have been reported, with an aim to simultaneously address the two largest micronutrient deficiencies, which affect some 2 billion people worldwide. Indian scientists from the

National Institute of Nutrition (NIN) first initiated research on double fortification of salt and made prominent contributions to this area (Mannar et al., 1989; Narasinga Rao, 1990). However, the formulations developed in these early studies were not technically or commercially feasible (Diosady et al., 2002a).

The technical challenges in developing double fortified salt are the instability of iodine and undesirable organoleptic properties of bioavailable, stable iron compounds. Ferrous compounds can react with iodate salts, resulting in the loss of iodine and reduced iron bioavailability (Diosady et al., 2002b)

The Food Engineering Research Group at University of Toronto has been active in developing microencapsulation-based technologies to prevent this reaction in double fortified salt. Ferrous fumarate was selected as the optimal iron source due to its relatively high bioavailability, stability, and bland taste. It was first agglomerated to match the size of salt grains, and then colour-masked and encapsulated using soy stearine, or other hydrophobic polymers (Diosady, 2007). The resulting iron premix can be blended into iodized salt at an appropriate ratio to achieve target nutritional levels of both iodine and iron. This formulation has been scaled up to allow commercial production, and the technology was transferred to two pharmaceutical manufacturers in India. The double fortified salt was highly efficacious in Indian field trials showing significant improvements in the iron and iodine status of over 1 million children (Andersson et al., 2008)

The process, though effective, produced irregularly shaped porous particles with low density and limited mechanical strength.
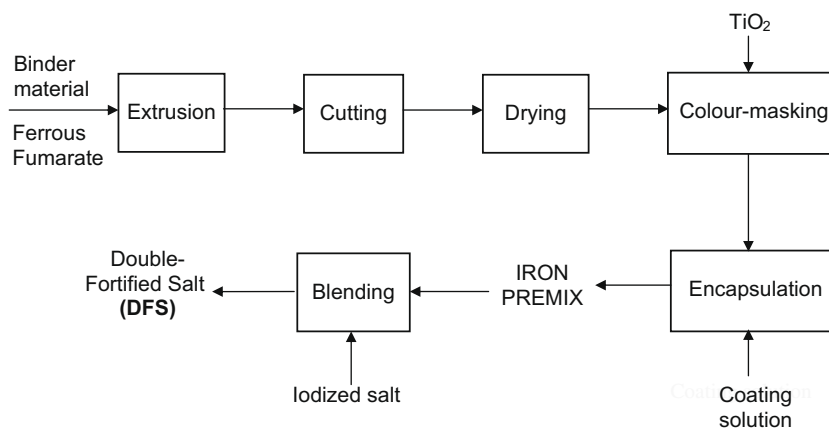
Fig. 1. Schematic process flow for making microencapsulated ferrous fumarate.

Accordingly we embarked on a product improvement program aimed at obtaining denser particles with desired colour and surface appearance, while reducing processing complexity and both capital equipment and operating costs. As the first step in completely revising the process an extrusion-based process for agglomerating ferrous fumarate has been developed. The process flow diagram is presented in Fig. 1.

In the present technique extrusion was used to agglomerate ferrous fumarate powder to salt-sized particles, which were then colour-masked and encapsulated by an edible polymer. The development work resulted in an extruded, colour-masked, and polymer coated ferrous fumarate premix formulation which had much improved physical characteristics in terms of shape, size, colour, texture, and density compared to the premix produced by the technique of fluidized-bed agglomeration and lipid coating (Li et al., submitted for publication). Double fortified salt (DFS) produced with the iron premix made by this process was expected to have improved chemical stability and high bioavailability, while reducing the cost of delivering iron to the consumers.

The objective of the present study was to test the stability of DFS produced with this premix, in an effort to confirm the central role of physical separation of iodine and iron in stabilizing iodine in DFS and also to gain better insight into iodine degradation in DFS at high temperature and humidity. Ferrous fumarate premixes produced using the best formulations were blended into iodized salt to form double fortified salt (DFS). Their storage stability was followed for one year at 40 °C and 60% relative humidity (RH). The storage conditions were selected to approximate the extremes of heat and humidity that the product may experience in tropical regions, where the salt double fortification technology is most needed. This paper reports the effect of different stages of processing on the storage stability of iodine and ferrous iron, and interprets the results in terms of the interaction between iodine and ferrous iron in DFS.

2. Materials and methods

Food-grade ferrous fumarate, particle size of ~50 μm, was obtained from Dr. Paul Lohmann Chemicals, Germany. The binder material used in the study, durum wheat flour, was procured from a local supermarket. It was one of the better binders identified in the earlier study (Li et al., submitted for publication). TiO$_2$ was used as the colour-masking agent at a coating level of 25% (w/w) of the extruded iron agglomerates, based on preliminary tests (Yadava, 2008). We found several HPMC-based polymers that were effective in coating the premix particles. Accordingly, Methocel E6 (Hydroxypropyl Methylcellulose obtained from Dow Chemicals

Co., USA) was used in this study to produce final iron premixes. Other formulation components and analytical reagents were used at food-grade or analytical grade, and obtained from Sigma–Aldrich, Canada. Food-grade, iodized salt (0.01% I$_2$ from potassium iodate) was provided by Kensalt, Kenya through the Micronutrient Initiative (MI).

2.1. Microencapsulated iron premix formulation preparation

The detailed procedures for preparing the microencapsulated iron premix, consisting of extrusion, colour-masking, and encapsulation, were reported earlier (Li et al., submitted for publication). The formulations used in this study are presented in Table 1. Specifically, two extruded, two colour-masked, and three microencapsulated iron particles were prepared using the optimal formulation and operation parameters reported earlier.

2.2. DFS sample preparation and stability test

Kenyan iodized salt with a nominal iodine concentration of 100 mg/kg (ppm) was used to prepare the double fortified salt samples. The formulated iron particles characterized in Table 1 were added into this salt at a ratio calculated to produce 1000 ppm iron in the DFS. The calculated ratio of premix to salt ranged from 1:160 to 1:200 depending on the actual iron content in the premix formulations. An untreated ferrous fumarate control sample was also prepared by adding ferrous fumarate powder directly to the iodized salt. The iron premix prepared on an industrial scale by Glatt Air Technology, Seattle, USA, using fluidized-bed agglomeration and lipid coating was used as the baseline for the

Table 1
Microencapsulated ferrous fumarate formulation design.

Formulation # of iron particles and premixes	Extrusion (binder: durum flour) Binder: FeFum (weight ratio)	Colour-masking (dry adhesion) TiO$_2$ (% of extrudate mass)	Microencapsulation (encapsulant: Methocel E6) Encapsulant level (% of colour-masked extrudate mass)
E-1	30:70		
E-2	25:75		
C-3 (based on E-1)	30:70	25	
C-4 (based on E-2)	25:75	25	
P-5 (based on C-3)	30:70	25	15
P-6 (based on C-3)	30:70	25	10
P-7 (based on C-4)	25:75	25	5

proposed process improvement. The salt samples were stored in an environmental chamber at 40 °C and 60% relative humidity (RH) for one year and analyzed for iodine and ferrous iron at the beginning of the storage and after 1, 3, 6, and 12 months. The details of sample preparation are presented in Table 2.

2.3. Iodine analysis

Iodine content in DFS was determined by iodometric titration (AOAC method 33.149) based on the principle of reducing the iodate in the salt to free iodine, which can be titrated by sodium thiosulphate using starch as a colour indicator. The detailed procedure was reported by Diosady et al. (2002a).

2.4. Iron analysis

Total and ferrous iron content in DFS were determined by spectrophotometry (Harvey et al., 1955), as a complex with 1, 10-phenanthroline. The detailed procedure was reported by Oshinowo et al. (2004).

2.5. Iodine degradation kinetics

Iodine degradation in the DFS samples during the storage at 40 °C and 60% RH was presented by plotting the ln (% remaining) versus time (months). The first-order rate constants (k) and half-life ($t_{1/2}$) (time required for 50% of the iodine to disappear) were calculated.

2.6. Statistical analysis

Data from chemical assays were obtained from four replicates, and reported as the mean value ± standard deviation (SD). One-way ANOVA was used to examine the statistical significance between the sample performances, using an Origin Pro 7.5 Program Package.

3. Results and discussion

A total of ten samples of double fortified salt (DFS) were prepared and stored for an extended period of one year. To test the effectiveness of the different levels for processing the iron premix, the DFS samples were prepared with iodized salt and ferrous fumarate that was in one of the forms of (1) untreated, (2) extruded, (3) extruded and colour-masked, and finally (4) extruded, colour-masked and polymer coated. Two levels of iron addition were used in the preparation of the extruded samples. To check the background stability of iodine in the salt, the original iodized salt sample was also stored and tested for iodine retention.

As the motivation for the program has been to improve and simplify the currently used fluidized-bed agglomeration and lipid coating process, a sample of premix prepared some three years earlier by Glatt Air Technique, NJ, USA was also included in the study (Sample 10). The iodine and ferrous iron content of these samples during twelve months storage at elevated temperature and humidity are presented in Table 3.

Both ferrous iron and iodine were stable in DFS prepared with the well encapsulated, extruded premix. The best formulation (P-6) was prepared by extruding ferrous fumarate using 30% (w/w) durum wheat flour as binder. The extruded particles were "whitened" using 25% (w/w) of TiO_2 and finally coated with 10% (w/w) of a commercial hydrophilic polymer, Methocel E6 (HPMC) obtained from Dow Chemical Co., USA. After one-year storage the DFS made with this formulation lost less than 15% of the iodine and 10% of the ferrous iron originally added to the salt.

3.1. Iodine stability

In the original iodized salt, iodine (in the form of potassium iodate) was reduced by some 15% during the year. As expected the addition of untreated FeFum powder resulted in the complete loss of iodine after one year. This is not surprising considering the large surface area of the FeFum powder (~50 μm particle size) and the high moisture level, which promoted the reaction between the iodine and iron compound.

The uncoated, extruded particles also caused significant iodine loss, varying from ~50% to ~65%, depending on the amount of the binder used in the extrusion formulation. When less binder
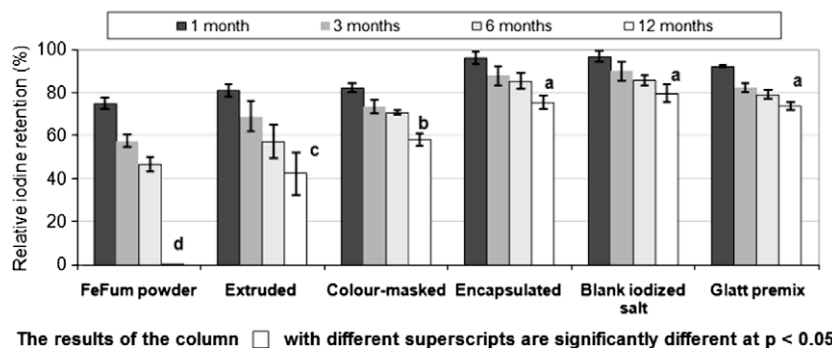
Table 2
Double fortified salt (DFS) samples prepared for the one-year storage stability test.

Sample #	Premix/control added
1	Blank iodized salt (no iron added)
2	Ferrous fumarate powder without treatment
3	E-1 (extruded particles made with durum flour: FeFum = 30:70)
4	E-2 (extruded particles made with durum flour: FeFum = 25:75)
5	C-3 (particles based on E-1 was colour-masked with 25% TiO$_2$ by dry adhesion)
6	C-4 (particles based on E-2 was colour-masked with 25% TiO$_2$ by dry adhesion)
7	P-5 (final iron premix based on C-3 was coated with 15% of Methocel E6)
8	P-6 (final iron premix based on C-3 was coated with 10% of Methocel E6)
9	P-7 (final iron premix based on C-4 was coated with 5% of Methocel E6)
10	Glatt iron premix prepared by commercial process based on the fluidized-bed agglomeration and lipid coating

Table 3
Iodine and ferrous iron retentions in DFS samples after one-year storage at 40 °C and 60% RH.

Sample #	Premix/control added	Iodine retention (ppm) (initial iodine = 106.4 ppm)	Ferrous iron retention (ppm) (initial ferrous iron = 1000–1100 ppm)
1	Blank iodized salt (no iron added)	84.7 ± 4.2	–
2	Ferrous fumarate powder	0	835.3 ± 54.5
3	E-1 (extruded particles 30:70)	52.7 ± 0.5	897.3 ± 10.8
4	E-2 (extruded particles 25:75)	37.9 ± 0.4	870.5 ± 19.9
5	C-3 (colour-masked particles based on E-1)	63.8 ± 1.5	920.2 ± 19.4
6	C-4 (colour-masked particles based on E-2)	60.1 ± 4.0	915.9 ± 48.8
7	P-5 (final iron premix based on C-3 with 15% coating)	79.5 ± 2.1	1079.4 ± 18.4
8	P-6 (final iron premix based on C-3 with 10% coating)	83.9 ± 1.6	1061.2 ± 8.9
9	P-7 (final iron premix based on C-4 with 5% coating)	77.2 ± 1.2	1059.9 ± 78.6
10	Glatt iron premix prepared by fluidized-bed agglomeration and lipid coating	78.6 ± 1.9	996.8 ± 21.4

Note: the results are mean ± standard deviation of four replicates for each measurement.

Fig. 2. Iodine stability in DFS samples containing FeFum in various forms – untreated powder, extruded, colour-masked, and microencapsulated formulations, after one-year storage at 40 °C and 60% RH. (*Note:* the results are the mean values obtained from four replicates, and the error bars represent the standard deviations.)

was used (E-2 vs. E-1), the iron was more exposed in the particles, resulting in more more iodine loss in the fortified salt samples. Clearly, extrusion of ferrous fumarate was inadequate to prevent iodine loss. It mainly served the purpose of bringing the particle size of the iron compound (ferrous fumarate powder) to match the salt grains.

Colour-masking by TiO_2 provided a physical barrier surrounding the extruded particles, which resulted in higher iodine retentions. This was more pronounced in the sample made with the higher iron content (C-4), where the iodine retention increased from ∼38% to ∼60%. This was a somewhat unexpected benefit, as the role of TiO_2 was to mask the reddish colour of ferrous fumarate, making the iron premix less visible in the white DFS.

All polymer coated FeFum premixes retained >70% of the iodine in DFS after one-year storage. Specifically, P-5 and P-6 coated with 15% and 10% of the polymer, respectively, retained ∼75% and ∼80% of the original iodine. P-7 coated with only 5% of HPMC had an iodine retention of 72%. The Glatt premix, which had a 40% of soy stearine coating, retained 74% of the iodine.

The results confirmed that the more compacted iron premix prepared by extrusion and hydrophilic polymer coating could effectively prevent the loss of iodine caused by direct interaction between iodine and iron, leading to high iodine stability in the DFS samples.

In order to clearly illustrate the effects of different process steps, the storage stability data obtained over time were grouped based on the levels of treatment and presented in Fig. 2. One-way ANOVA test was used to analyze the results. Clearly, each step of the process, i.e., extrusion, colour-masking, and polymer coating, had a significant positive effect on iodine stability. The iodine retention in the DFS made with the coated extrusion-based premixes was similar to that of the iron-free iodized salt, indicating that the premix was essentially inert in this system.

3.2. Ferrous iron stability

The retention of ferrous fumarate in its reduced ferrous form is the key to maintaining its high bioavailability. The conversion of ferrous to ferric iron is due to oxidation in the presence of oxidants, such as potassium iodate or oxygen. Thus, the encapsulation process is also needed for protecting the ferrous iron from oxidation in DFS.

As reported separately (Li et al., submitted for publication), the processing steps of extrusion, colour-masking, and polymer coating had little impact on ferrous oxidation, with >90% of the iron retained in its ferrous form after the premix production. After 10 months storage at ambient conditions, little further ferrous loss occurred in the absence of iodine, with <5% of loss in extruded particles, 3–4% of loss in extruded and colour-masked particles,

and <2% of loss in the microencapsulated premixes. However, when these iron particles were added into iodized salt and stored at higher T and RH, the ferrous iron loss was more pronounced after one-year storage: 3–4% in the encapsulated premixes, 7–10% in the colour-masked particles, and up to 14% in the extruded particles. Clearly each process step had a cumulative effect in the protection of not only iodine, but also ferrous iron.

To better understand the effects of the key parameters of the process, the data were grouped by process stages – extruded, colour-masked, and encapsulated – and analyzed using one-way ANOVA test, as shown in Fig. 3. Not surprisingly, the two encapsulated forms, newly lab-processed premixes coated with the hydrophilic polymer (HPMC) and the Glatt premix made by commercial-scale fluidized-bed agglomeration and soy stearine coating, had significantly higher ferrous iron retentions than the other iron particles ($p < 0.05$). It confirms that all coatings played an important role in protecting the stability of ferrous iron. Similarly, the colour-masking layer of TiO_2 also provided a physical barrier to the iron extrudates, which resulted in a less pronounced, but significant reduction in ferrous loss. Although the averaged value of ferrous retention in the extruded particles was greater than that of the iron powder, the difference between these two groups was not statistically significant ($p > 0.05$).

When the ferrous retention values were compared within each group over the storage period, it was noticed that ferrous iron was stable at the ambient conditions in the absence of iodine. In contrast, when added into the iodized salt and stored at higher T and RH, the untreated FeFum powder, the extruded, and the colour-masked particles experienced significant ferrous loss over the year. Clearly, both the storage conditions and the presence of iodine contributed to the ferrous loss.

The stability of the ferrous iron encapsulated in a readily digestible polymeric coating indicates that the bioavailability of iron will not be reduced by this technique. This conclusion was also supported by in vitro digestibility measurements by Li et al. (submitted for publication).

3.3. Iodine and iron interaction in DFS

Correlation analysis was used to confirm that the losses of iodine and iron in the DFS were related. As indicated below in Eqs. (1) and (2), ferrous iron compounds can reduce iodate, forming the volatile elemental iodine (Diosady et al., 2002a). Iodized salt loses iodine due to interactions with salt impurities, and the environment. The actual iodine loss due to interaction with iron was calculated by subtracting the iodine loss in the iron-free iodized salt from the loss of iodine in the DFS samples. Similarly, the ferrous iron loss in the DFS samples due to the iodine–iron interaction was calculated by taking into account the ferrous loss during stor-
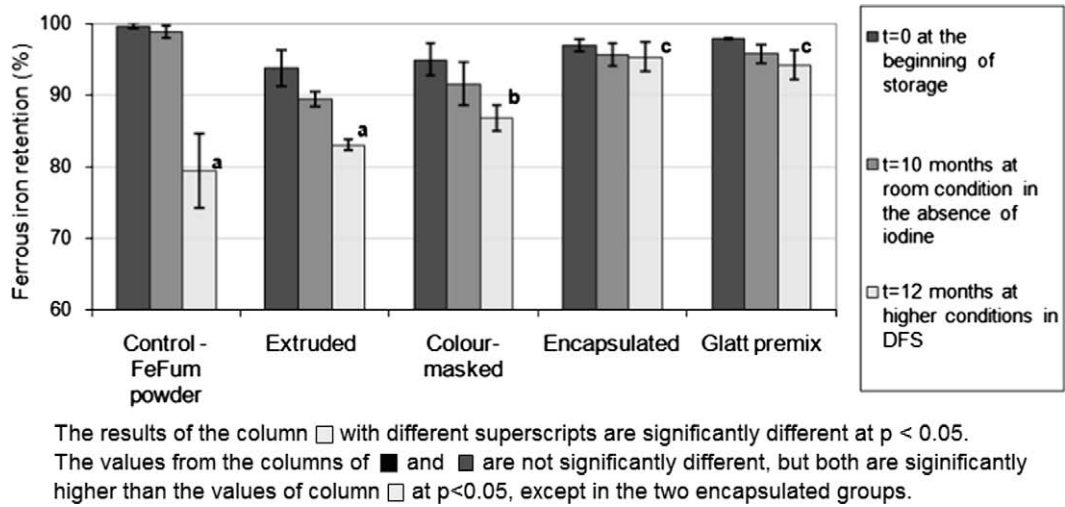
The results of the column □ with different superscripts are significantly different at p < 0.05.
The values from the columns of ■ and ▓ are not significantly different, but both are siginifically
higher than the values of column □ at p<0.05, except in the two encapsulated groups.

Fig. 3. Ferrous iron stability in various FeFum forms after 10 months storage at the ambient condition and in the DFS samples after one-year storage under 40 °C and 60% RH, respectively. (*Note:* the results are the mean values obtained from four replicates, and the error bars represent the standard deviations.)
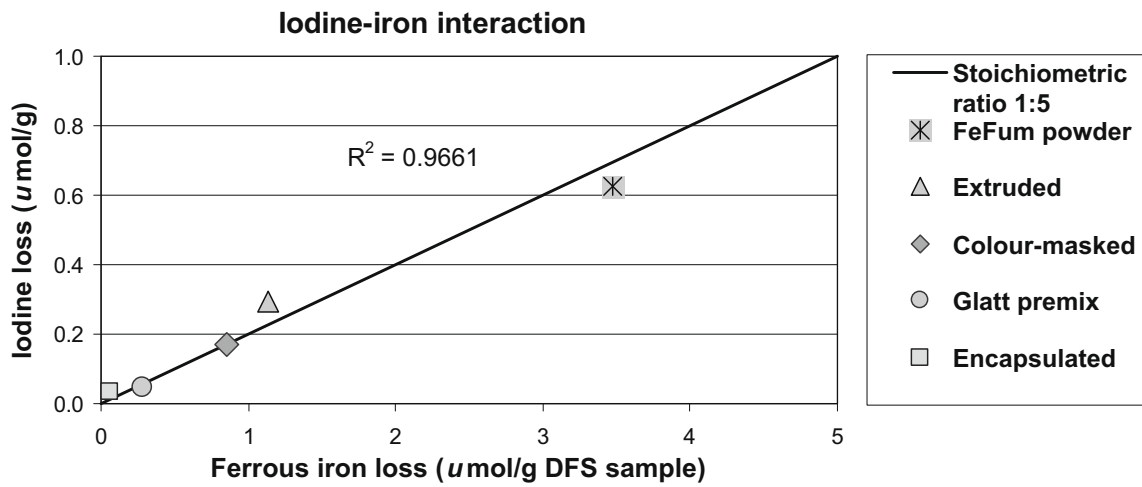

Fig. 4. Iodine–iron interaction in DFS – correlation analysis between iodine and ferrous iron losses in the DFS samples containing variously prepared ferrous fumarate particles after one-year storage at 40 °C and 60% RH.
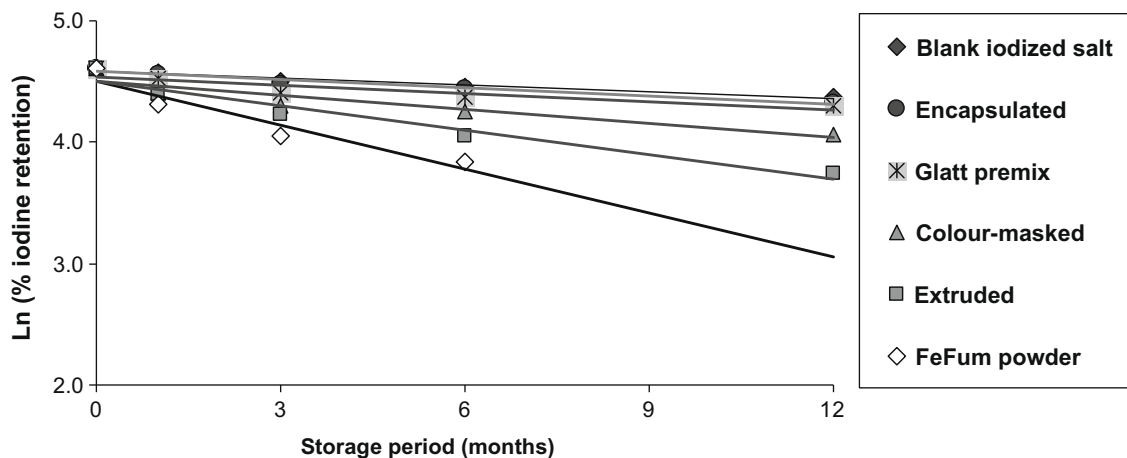

Fig. 5. Apparent first-order degradation kinetics of iodine in the DFS samples made with processed ferrous fumarate particles during one-year storage under 40 °C and 60% RH.

Table 4
Experimental data processed for correlation analysis of the iodine–iron interaction in DFS.

	Losses in mass (μg/g DFS) after one-year storage due to the interaction		Losses in moles (corrected by taking into account the atomic weights of iron and iodine, 56 and 127, respectively)	
	Ferrous iron (μg/g)	Iodine (μg/g)	Ferrous iron (μmol/g DFS salt)	Iodine (μmol/g DFS salt)
FeFum powder (no treatment)	194.6	79.42	3.48	0.63
Extruded	63.5	37.39	1.13	0.29
Colour-masked	47.7	21.45	0.85	0.17
Encapsulated	3.1	4.21	0.06	0.03
Glatt premix	15.6	5.76	0.28	0.05

Table 5
Iodine first-order degradation rate constant (k) and the storage half-life estimated for iodine in the DFS containing various ferrous fumarate particles, stored at 40 $^\circ$C and 60% RH.

DFS samples	Iodine degradation rate (month^{-1})	Iodine half-life (months)
Control-iron-free iodized salt	0.0185	36
Encapsulated FeFum premixes	0.0223	30
Glatt premix	0.0228	27
Colour-masked with TiO$_2$	0.0384	15
Extruded FeFum agglomerates	0.0666	9
FeFum powder	0.1202	5

age in the absence of iodine from each of the FeFum forms. A plot was then generated using the iodine loss values against the ferrous loss, as shown in Fig. 4. The points indicated the experimental data, while the straight line represented the stoichiometric ratio of iodate and ferrous iron according to the reaction represented by Eqs. (1) and (2), i.e., 1:5. The experimental mass loss data were recalculated as molar losses (μmol/g) by taking into consideration of the atomic weights of iodine and iron, 127 and 56, respectively (Table 4).

$$2KIO_3 + 10Fe^{2+} + 10H^+ \rightarrow I_2 + 10Fe^{3+} + 2KOH + 4H_2O, \quad (1)$$
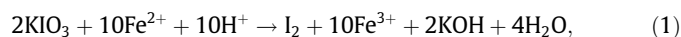
or expressed in terms of the reacting ions:

$$2I^{5+} + 10Fe^{2+} \rightarrow I_2 + 10Fe^{3+} \quad (2)$$

As shown in Fig. 4, the experimental results matched the theoretical values very well, confirming that the reaction of iodine with iron is the likely cause of the degradation of DFS samples. Each step of the microencapsulation process had positive effect on preventing the direct interaction, as shown with a simultaneously progressive reduction in losses of both iodine and ferrous iron. When examining the individual effect of each step, it is clear that the extrusion process had the greatest impact on reducing the loss of both nutrients due to the significant reduction in surface area when the iron powder was agglomerated into a more compacted form. The coating by inert TiO$_2$ enhanced this effect further due to the solid physical barrier it formed. The polymer coating was still needed to complete the barrier needed for stabilizing both nutrients.

3.4. Iodine degradation kinetics in DFS

The iodine retention in the DFS was plotted as ln (% iodine retention) vs. the storage time (Fig. 5). A good correlation between the data and the linear regression lines (with the correlation coefficients >0.9 for all sample groups) confirmed that the iodine degradation in the DFS followed apparent first-order kinetics.

The statistical analysis on the degradation trend lines suggested there was no significant difference ($p > 0.05$) between the two encapsulated groups and the control – the original iron-free iodized salt. It indicated that the incorporation of well-coated iron premixes in the iodized salt did not cause changes to iodine degradation patterns. However, the incorporation of untreated and partially processed ferrous fumarate particles had significant impacts on iodine degradation parameters, with progressively increased first-order rate constants and decreased half-lives, as shown in Table 5.

4. Conclusions

This study has demonstrated that a ferrous fumarate premix produced by extrusion agglomeration, colour-masking, and polymer coating, when incorporated in iodized salt produces an attractive double fortified salt (DFS) that is stable for an extended period at elevated temperature and humidity typical of the tropics, and is therefore an attractive vehicle for the simultaneous prevention of iodine deficiency disorders and iron deficiency anaemia.

Each step of the microencapsulation process contributed to producing an inert premix: extrusion significantly reduced the exposed surface area of ferrous fumarate, TiO$_2$ formed a water-insoluble physical barrier that filled the surface pores of the extrudate, and the polymer over-coating film prevented the diffusion of smaller molecules (such as water and iodate) into the FeFum core. These effects collectively resulted in significantly reduced interaction between the two species, subsequently enhanced stability of iodine and ferrous iron in the DFS.

During stability tests of double fortified salt, iodine loss through conversion to elemental iodine and subsequent sublimation, and ferrous iron oxidation followed the stoichiometric ratio of a direct reaction between these two micronutrients, confirming the need for physical separation between them in a fortified food product, such as DFS. Iodine loss in iodized salt followed apparent first-order kinetics. The presence of iron without sufficient coating accelerated the iodine loss, as expected. However, the DFS made with well encapsulated FeFum premix was estimated to have similar shelf-life to iodized salt. This clearly demonstrates that double fortification of salt prepared using this technology will be a viable approach to delivering these two micronutrients through the normal salt distribution system.

The best formulation (P-6) prepared by extruding ferrous fumarate using 30% (w/w) durum wheat flour as binder, colour-masked with 25% (w/w) of TiO$_2$ and finally coated with 10% (w/w) HPMC results in stable double fortified salt that is ready for pilot scale testing.

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